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THE REDUCING ENZYMES.

BY M-EMM. POZZI-ESCOT.

The reducing enzymes were discovered by J. de Rey-Pailhade, and he was the only one to occupy himself with this question up to the end of the year 1901, when, at his suggestion, I undertook the study. My first communication upon that subject to the Academy of Sciences of Paris bears the date January 6, 1902.¹

For a long time the existence of reducing enzymes was denied. Their reality is above suspicion, and at the present time no one has any doubts about it. De Rey-Pailhade even believes—and he will pardon me for making public this unpublished part of his correspondence—that there are "reduc-tases" and also "hydrogenases." I agree with him fully, but am inclined to believe that there is but one large family in which is a group of individuals whose reducing function is also to add hydrogen (hydrogenize). It is an idea somewhat difficult to explain, and since we lack definite facts to establish it, I shally simply mention it.

De Rey-Pailhade gave the name "philothion" to the en-

¹ M-Emm. Pozzi-Escot: "Contribution à l'étude du philothion," *Compt. rend.*, 134, 66 (1902).

zyme discovered by him. In 1885, in an investigation undertaken to determine the mechanism of the elimination, in the organism, of free sulphur taken into the gastro-intestinal tract, he was led to the conclusion that there is direct combination of this sulphur with nascent hydrogen of fermentation with the formation of hydrogen sulphide.¹ The next year, 1886, he communicated to the Society of Natural History of Toulouse the result of experiments that led him to admit the existence, in living cells, of an organic substance that could form hydrogen sulphide when in contact with free sulphur,² but this was only his personal conviction without positive proofs to support it. It was necessary to isolate this active substance, to extract it from the cells containing it, to show that it actually had the characteristics of an enzyme. He made a large number of unsuccessful experiments before reaching this result, but finally, in 1888, he showed that an active substance may be extracted from beer yeast by means of alcohol.³

Nature of Philothion.

Philothion, like all other enzymes, must be a molecular complex possessing active affinities and charged with a relatively large amount of chemical energy. Its essential characteristic is to form hydrogen sulphide from free sulphur. De Rey-Pailhade has been led to regard it as a special hydrogenized

¹ J. de Rey-Pailhade: "Sur le formation de l'hydrogène sulfuré dans l'organisme à la suite de l'ingestion de quelques médicaments; Thèse pour le doctorat de médecine, Montpellier, (1885)." Bull. Soc. Hist. Nat. Toulouse, May 8, 1885.

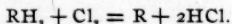
² J. de Rey-Pailhade: "Recherches expérimentales pour expliquer l'absorption du soufre par la voie gastro-intestinale," Bull. Soc. d'Hist. Nat. Toulouse, 1886, p. 116.

³ J. de Rey-Pailhade: "Sur un corps d'origine organique hydrogénéant le soufre à froid," Compt. rend., June 11, 1888; "Nouvelles recherches physiologiques sur la substance organique hydrogénéant le soufre à froid," *Ibid.*, July 2, 1888; "Sur un nouveau principe immédiat, le philothion, et sur sa propriété d'hydrogéner le soufre," Bull. Soc. Hist. Nat. Toulouse, July 4, 1888.

About the same time that de Rey-Pailhade made known philothion and its fundamental property, M. Olivier, of the Revue Générale de Sciences, announced as a great novelty and an important physiological discovery, that the organic matter of the "sulphuraires" (*Oscillaria*, *Ulothrix*, and *Beggiatoa*, spp.) yields hydrogen sulphide with free sulphur. It was June 11, 1888, that de Rey-Pailhade made known philothion and its remarkable property. The note of Olivier is dated June 8, but it was not published until June 18. The priority of de Rey-Pailhade is incontestable. Compt. rend., June 11, 18, 25, July 2, 16, and Aug. 13, 1888.

compound of the formula RH_2 . R is here a radical, of as yet unknown composition, to which 2 atoms of hydrogen are loosely joined in much the same way as the 2 atoms of hydrogen that differentiate indigo-blue from indigo-white.¹ This would explain the action of reagents towards philothion.

Take, for example, the action of the halogens. Chlorine, bromine, and iodine destroy philothion instantaneously, an action which is due, according to de Rey-Pailhade, to a sudden removal of the hydrogen, as indicated in the following theoretical equation :



I do not wish to deny the explanation of de Rey-Pailhade, but may state that it is hypothetical, and he has never pretended to give it the value of a positive fact.

Preparation of an Active Hydrogenizing Liquid.

De Rey-Pailhade, at the beginning of my investigation, gave me the following method for the preparation of a solution of philothion. Treat 100 grams of cold, pressed top yeast with 55 grams of water and 45 grams of 90 per cent alcohol. Fill a bottle completely with the mixture, cork it, and shake for twenty-four hours at the ordinary temperature. Then filter, twice if necessary.

By following these directions one may, indeed, obtain a solution capable of hydrogenizing action, but I have found it necessary to modify the mode of operation in order to obtain more actively hydrogenizing solutions. At first I treated 1000 grams of the bottom yeast of beer, which is richer in reducing enzymes than top yeast, with 500 grams of 50 per cent alcohol, to which has been added a small quantity of an antiseptic, such as sodium fluoride, thymol, etc.

There are obtained much more active solutions by causing the plasmolysis of the dry yeast cells by means of some solid substance. De Rey-Pailhade uses sodium chloride,² but the

¹ This formula must be far from the truth. It seems to me much more logical to suppose that the hydrogen is not free, but is closely united to a radical, R, in somewhat the same way as the hydrogen of the aldehyde group.

² Congrès de Turin, Sept., 1901.

process that gives the best result is the following: Treat 500 grams of compressed yeast with 100 to 150 grams of powdered saccharose, and triturate the cooled mass. Soon the plasmolysis becomes quite active, producing complete liquefaction of the mass, which is then transferred to a precipitating vessel. A little thymol is added, and the whole is left for twelve hours. During this time the sucrase and zymase of the yeast act on the saccharose, causing exceedingly active fermentation with lively effervescence of the entire mass.¹ When the action is over, add a little water and filter. The solution is very active.

I have also obtained good results with pure glycerol. My experiments have shown that yeast that has developed in contact with the air is much poorer in the reducing enzyme than that which has led an anaerobic life. Hence bread yeast is much poorer than the bottom yeast of beer.

The liquid obtained by these different methods is slightly acid, a liter of it being equivalent to 1 to 3 grams of sulphuric acid. It is most active when in this condition. An exceedingly active solution may be obtained by precipitating the enzyme and redissolving it in a small quantity of water.²

General Properties of Philothion.

The active agent in the solution mentioned above was named philothion by de Rey-Pailhade.

The action of philothion upon oxygen³ is especially impor-

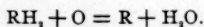
¹ I have been asked for a proof of the existence of zymase. I find it in the fact that in the presence of an enormous excess of antiseptic, sufficient to kill every cell, the liquid obtained in the manner stated can change into alcohol as much sugar as the corresponding amount of yeast, and in a shorter time. It is well to state that this procedure permits the study of Buchner's zymase.

² It is difficult to precipitate the reductases by alcohol since it requires such an enormous quantity of the latter. I prefer ammonium or magnesium sulphate, or the method of Cohnheim or of Danilewski. See Pozzi-Escot: "Les diastases et leurs applications," pp. 58-59.

³ J. de Rey-Pailhade: "L'oxydation de l'extrait alcoolique de levûre de bière," Bull. Soc. Hist. Nat. Toulouse, 27 Mars et Mai (1889); "Recherches expérimentales sur le philothion, principe immédiat répandu dans les deux règnes vivants, son rôle physiologique probable dans l'absorption de l'oxygène par la cellule vivante." Brochure in 8°, Mars (1891). Masson, éditeur, Paris; "Faits pour servir à l'histoire des oxydations intraorganiques, montrant l'absorption de l'oxygène," Bull. Soc. Hist. Nat. Toulouse, 7 Dec. (1892); "Nouvelles recherches sur le philothion, son rôle physiologique dans les oxydations intraorganiques, Congrès des Sociétés Savantes,

tant, and has caused much active discussion. Oxygen combines with philothion at the ordinary temperature, according to de Rey-Pailhade, who presents the following facts as sufficient proof for this statement: If an alcoholic solution of philothion, prepared as above, is exposed to the air, it loses its activity as an enzyme, after a certain time. On the other hand, if one performs a similar experiment with the same liquid, but removes the oxygen from the solution and the space above it by means of a current of pure hydrogen, nitrogen, or carbon dioxide, the extract retains its activity for a much longer time.

One may easily demonstrate the absorption and the hydrogenation of the oxygen dissolved in the extract of philothion by making a series of determinations of the dissolved oxygen by the method of Schützenberger.¹ The action of oxygen upon philothion may be represented thus:



R has the meaning given it in the formula of de Rey-Pailhade.

Philothion exists in most living cells, but the rôle played by oxygen in these organisms is known, so that one may well be surprised at finding philothion in them. Evidently, if the above-mentioned destruction is effective, and not a mere specu-

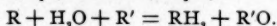
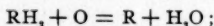
(1892)," *Bull. Soc. Hist. Nat. Toulouse*, p. 43 (1892); "Sur l'absorption de l'oxygène par les êtres vivants," *Bull. Soc. Hist. Nat. Toulouse*, 4 Mai (1893); "Mémoire sur l'absorption de l'oxygène par les tissus vivants, Congrès pour l'Avancement des Sciences, Besançon (1893)," *Bull. Soc. Hist. Nat. Toulouse*, p. 57 (1893); "Sur l'existence de deux ferments d'oxydation dans les plantes, Congrès pour l'Avancement des Sciences de Carthage (1896)," *Compt. rend. soc. biol.*, 11 Mai (1896); "Sur l'oxydation des tissus," *Bull. Soc. Hist. Nat. Toulouse*, 2 Juin (1897); "Oxydations et réductions organiques," *Ibid.*, 7 Juillet (1897).

M-Emm. Pozzi-Escot: "Nouvelles recherches sur les principes réducteurs des tissus vivants," *Compt. rend. de l'Académie de Médecine*, 25 Mars (1902); "Principes réducteurs des tissus vivants," *Soc. Médecine de Gand*, 5 Juin (1902).

¹ The preceding facts were established by de Rey-Pailhade. I have shown that philothion, which must be a mixture of two reductases, is not always destroyed as rapidly as seems to be the case from his experiments. I have been able to keep in the laboratory, in a partly filled flask, a solution of philothion containing thymol from December 23 to the end of February. I must admit that it had then lost the property of hydrogenizing sulphur, but it still acted upon hydrogen peroxide, on indigo-carmin, and on tincture of guaiacum, all of which, as will be seen later, are characteristic reactions of a reductase. The facts seem to show that jacquemase is contained in philothion.

lation, there must occur simultaneously the destruction and regeneration of philothion.

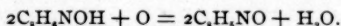
The following is the explanation which is suggested by de Rey-Pailhade: When, in the organism, the philothion, RH_2 , has been changed into R by combining with oxygen to form water, it returns to the condition RH_2 by the simultaneous action of R and of an oxidizable substance, R' , upon a molecule of water. The water is dissociated, the hydrogen going to R to re-form RH_2 , and the oxygen combines with the oxidizable substance, R' :



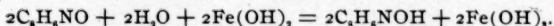
These equations represent a fact which must be regarded as certain until further information is obtained. We see, then, that philothion, together with water, acts as a carrier by means of which free oxygen is brought to the oxidizable substance. That is the part played by an enzyme, a chemical agent formed entirely by a living cell, and finally working for its benefit. (It is true that there are many enzymes that do not yet come under this definition, which, however, holds for certain groups of well-known diastatic ferments.) The quantity of oxygen absorbed in this indirect way appears to be important. The immediate regeneration of this principle by a phenomenon of hydrogenation is not only probable, but it is not without a parallel. It is known from the work of Armand Gautier that, in one part of the living cells of large animals the albuminoid tissues decay, when kept from the air, owing to the action of putrefying ferments. Thus the researches of Schloesing prove irrefutably that during the methane-fermentation of manure, water is decomposed into its elements. The oxygen combines with certain substances, and the hydrogen unites with carbon to form methane.¹

¹ Schloesing (père et fils): *Ann. Agro.*, 18, 5 (1893); *Compt. rend.*, 109, 835 (1889). Reiset: *Ibid.*, 108, 708, 779 (1888). Deherain: *Ann. Agro.*, 10, 395. Gayon: *Compt. rend.*, 98, 528 (1884). Behrens: *Centralbl. für Bakt.*, 3, 584, 639, 783 (1897). It must be remembered that nothing absolutely definite is known of the methane-fermentation, although it is so common, and also very important, for to it is due the breaking-down of most of the cellulose produced each year on the earth's surface. An important chapter on this subject is contained in Duclaux's *Traité de Microbiologie*, 4, 460.

We may approach the subject of the action of philothion upon oxygen in another way. It is known that indigo decomposes water, in the presence of a large number of oxidizable substances, in slightly alkaline solution. The indigo-white, in contact with oxygen, yields indigotin according to the equation :



On the other hand, indigo-carmine, when in contact with water and ferrous oxide, acts according to the equation :



Comparing these two equations with those given above for philothion, we see that they are similar. Of course, the resemblance is only accidental, but it is not without value.¹

I have taken up this theoretical part about the properties of philothion, and have obtained certain practical results of great interest that give a new idea of the rôle played by the reductases in the phenomena of oxidation taking place in the midst of living tissues (this work was brought before the Académie de Médecine of Paris by Armand Gautier, March 25, 1902).

I begin by preparing a very active solution of philothion and add to it 1 to 2 cc. of salicylic aldehyde, then the half-filled flask is closed with a ground-glass stopper and the mixture shaken, in contact with oxygen, for twelve hours. At the end of this time the liquid contains salicylic acid, which is detected as follows: The liquid is heated to boiling and the precipitated albuminoids are filtered off. Sodium carbonate is then added and the solution evaporated to dryness. The residue is dissolved in water containing hydrochloric acid and extracted with ether. The ethereal solution, when evaporated, yields crystals of salicylic acid, if any has been formed. This was the case in one experiment carried out in this way.

¹ De Rey-Pailhade: "Rôle du philothion et de la laccase en germination," *Compt. rend.*, 121, 1162 (1885); "Sur l'existence simultanée, dans les tissus animaux, du philothion et de l'oxydase chargée de l'oxyder," *Compt. rend. Soc. Biol.*, April 3 (1897); "Sur l'oxydation des tissus," *Bull. Soc. Hist. Nat. Toulouse*, 108, 3 (1897).

M.-Emm. Pozzi-Ricot: "Contribution à l'étude du philothion," *Compt. rend.*, 134, 66; "Contribution à l'étude de la casse," *Le Progrès Agr. et Vitic. de Montpellier*, March 9, 1902, p. 302.

In another series of experiments, I placed in contact with the hydrogenase some easily reduced substance containing oxygen, such as an iodate or arseniate, and found that the oxidation of salicylic aldehyde took place, apparently more actively. But too much value must not be attached to the determinations made under these conditions.

I do not pretend, as one might think, that philothion can act as an oxidase, but only that it may cause oxidation as a secondary reaction, accompanying the reduction, as the preceding experiments certainly prove. Hence, the theory of Hoppe-Seyler is verified.¹

It may be noted that the above-mentioned oxidation may, perhaps, not be due to the fixation of oxygen, but to the loss of hydrogen, and, as A. Étard has shown, this is not because the oxidizing agents are strong or weak, but is due to combinations and decompositions depending on the relative structures of the oxidizing agent and the substance oxidized.²

This action of oxygen on philothion is of very great importance; in the future it may play a leading part in our knowledge of the functions of the organism. We may say with de Rey-Pailhade that philothion, or more exactly, the reductases in the living cell are the gate, or one of the gates, through which free oxygen penetrates into the living cellular edifice. The tissues that use up much oxygen contain much philothion, or in other words, have a large gate for the entry of this indispensable aliment.

Reciprocal Action of Oxidases and Reductases.³

The reciprocal action of the oxidases and reductases is of

¹ M-Emm. Pozzi-Escot: "Les oxydases de la levûre," *Revue Oenophile*, 19, No. 4, p. 123 (1902), Bordeaux; Armand Gautier: "Leçons de Chimie biologique," 2^e Ed., p. 744.

² A. Étard: "De l'oxydation par voie de déshydrogénation au moyen des ferrocyanures: Oxydation du camphre," *Compt. rend.*, 130, 569 (1900).

³ De Rey-Pailhade: "Rôle du philothion et de la laccase dans les graines en germination," *Compt. rend.*, 121, 1162 (1895); "Sur l'existence simultanée dans les tissus vivant animaux du philothion et de l'oxydase chargée de l'oxyder," *Compt. rend. Soc. Biol.*, April 30, 1897; "Sur l'oxydation des tissus," *Bull. Soc. Hist. Nat. Toulouse*, 108, 3 (1897).

M-Emm. Pozzi-Escot: "Contribution à l'étude du philothion," *Compt. rend.*, 134, 66; "Contribution à l'étude de la casse, Le Progrès Agricole et Viticole de Montpellier," March 9, 1902, p. 302; "Sur le commensalisme de certains ferments oxydants et réducteurs dans certains tubercules," *Compt. rend.*, 134, 1006 (1902).

the greatest interest. Although we are not yet certain as to the mechanism of that action, we shall show what results have been arrived at. At first sight it seems as if the oxidases and reductases must be antagonistic, and experiment has shown that this is actually the case.

What should result from this antagonism where there are in the same cell, or in adjacent cells, secretions that oxidize, and others that reduce? It seems logical to admit that the labile elements of the two secretions will act reciprocally upon one another, there being a combustion of the hydrogen of the hydrogenase by the oxygen of the oxidase, and that this action will take place until one of the secretions is destroyed, leaving the field free for the action of the other. But these ideas are only *à priori*. It might be asked whether they are true, and especially whether they can be verified by experiment. De Rey-Pailhade has shown that when a tissue containing a reductase and an oxidase is finely ground, the oxidase seems to destroy the reductase by carrying the exterior oxygen, so that at the end of several hours only the oxidase is to be found, especially if the mixture be agitated in the air. Similarly, de Rey-Pailhade showed that the philothion in a germinating seed is often destroyed by the oxidase. According to his theory, the oxidases act on the hydrogenases, bringing about their destruction by combustion.

Admitting this to be true, it might still be asked whether the opposite might not be true also.¹ I have arrived at the following result: If, under suitable conditions, we allow a reductase to act on an oxidase, the reductase being in great excess, the oxidase may become "paralyzed" in its action. It is preferable to wait before penetrating the mechanism of this phenomenon, and giving a logical explanation of it.

De Rey-Pailhade has spoken of combustion; this must be inexact. I am inclined to believe that there is a momentary combination of the two enzymes, and that the unstable compound thus formed—whose composition and nature escape us—by means of some new adhesive process fixes itself in insoluble form upon the solid substances with which it comes in contact, and is thus eliminated temporarily.

¹ M-Emm. Pozzi-Escot: Bull. soc. chim. de Paris, [3], 27, 346 (1902).

The following is an unpublished experiment relating to this subject: The tubers of the dahlia (*Dahlia variabilis*), of the potato (*Solanum tuberosum*), and the Jerusalem artichoke (*Helianthus tuberosus*), contain oxidases. Sections of these tubers turn blue under the influence of an alcoholic tincture of gum guaiacum when exposed to the air. This reaction is of great interest and should be more closely studied. At first there is no change, then the blue is formed and disappears, and finally, at the end of a minute, the reaction suddenly acquires great intensity. This depends on the existence of oxidizing and reducing enzymes in the tubers. We may go still further in this way and cause solutions of a reductase and an oxidase to react upon one another. De Rey-Pailhade and I have accomplished this by experimenting in different ways. The experiment of de Rey-Pailhade follows: A solution of philothion is prepared by suspending compressed beer yeast in water containing 1.5 per cent of sodium fluoride. The mixture is put into a flask completely filled and stoppered. It is shaken for two days and then filtered. This antiseptic liquid yields much hydrogen sulphide when treated with powdered sulphur at 35°. The oxidase is obtained by grinding the cotyledons of germinating chick-peas for eight days in their weight of alcohol at 25°. This pulp is quite suitable for experiments. Two mixtures are made, each containing 25 cc. of the solution of philothion and 5 cc. of the pulp containing the oxidase. The first, A, is put into a flask of 150 cc. capacity, and the second, B, into a small bottle, which must be completely filled and well stoppered. A third mixture, C, made of 25 cc. of philothion and 5 cc. of oxidase which has been heated high enough to destroy this substance, is put into a 250 cc. flask.

The three flasks are mechanically shaken at the same time for five hours. When the liquid is then examined, it is found that A no longer contains philothion, although B and C contain a considerable quantity of it. The destruction of the philothion in the mixture C, where only the oxygen of the air acted, does not take place until after six or seven days, and it is found that the oxygen has almost entirely disappeared, and is replaced by carbon dioxide.

These experiments of de Rey-Pailhade seem to have established the following :¹ (1) That under the combined influence of the oxidase and oxygen, philothion is oxidized in a few hours ; (2) that by the action of oxidase alone, philothion is not destroyed ; (3) that by the action of oxygen alone philothion is slowly oxidized.

Animal tissues always contain simultaneously both oxidase and philothion, so that, in contact with the air, the philothion is rapidly destroyed, and hence plays a greater or less part in the respiration of the tissues.

I have sought for the mechanism of these phenomena, and, as mentioned above, have studied the potato, commencing with an investigation of the oxidases contained in it. I have shown that, although spread throughout the tuber, they do not freely exercise their oxidizing action except in the part near the exterior, while at the center their influence is moderated by the influence of the reducing enzymes that also exist there. This has led me to the idea of the "commensalism" of the oxidizing and reducing ferments.

Abelous and Biarnès have also studied the oxidases of the potato, but without result.² They showed that the juice of potatoes, extracted by pressure and precipitated by alcohol, yields a precipitate which, when redissolved, cannot oxidize salicylic aldehyde, in either neutral, alkaline, or acid solution. They therefore conclude that "there is reason to believe that the laccase of certain plants does not oxidize salicylic aldehyde, and, consequently, the soluble ferment which causes the oxidation of salicylic aldehyde is not the same as the laccase studied by Bertrand."³

Potatoes contain oxidases and reductases, but, in the presence of air, that is, of oxygen, the oxidizing action preponderates, the reducing action being destroyed. This can be

¹ De Rey-Pailhade has really not shown that the destruction of the philothion was due solely to the action of the oxidase. Was it really an oxidizing enzyme that destroyed the philothion, and has the philothion really been destroyed?

² E. Barbier : Thèse de médecine, Toulouse, No. 122, p. 89, 1895-96. If these authors could not obtain any oxidizing action, it is because equilibrium was established between the oxidizing action of the oxidase and the opposite action of the reducing enzyme.

³ "Contribution à l'étude des phénomènes oxydasiques dans les boissons fermentées," *L'Oenophile*, Feb., 1902, pp. 63-65.

shown as follows: Fill a long test-tube with bits of potato, and then pour upon them a dilute solution of indigo. Some of the upper pieces of potato are to be left uncovered by the liquid, and the whole is allowed to stand for twenty-four hours. It is then seen that the liquid is reduced at the bottom of the tube, is unchanged at the middle, and is oxidized at the part in contact with the air. The experiment is conclusive, but it does not always succeed.

The reciprocity of the action of the reductases and the oxidases has led me to suggest a new hypothesis regarding the disappearance of the oxidases of fruits during the alcoholic fermentation.¹

In fruits and vegetable substances used in the fermenting vats, there exist oxidases localized in special cells, and hydrogenases also situated in distinct cellular elements. The existence of oxidases in fruits, and especially in the grape, is certain from the result of experiments by Martinand,² and of Bouffard and Sémichon.³ It may be added that there are fruits that do not contain oxidases. The reductases also exist in fruits, as I have had occasion to show many times.

It follows that grape juice, before fermentation, generally contains enough oxidase to cause the ultimate breaking-down ("la casse") of the wine, yet it is known by experiment that a wine which has fermented normally is not generally subject to breaking down. All, or most of the oenoxidase of the original must is not found in the finished wine. Further, Alliot showed in 1901 that it is possible, by re-fermentation, to ameliorate and to render stable a wine that is apt to break down ("cassable").

By what means is the destruction of the oxidases, whose presence is so easy to show in the must, brought about, and why, in certain cases, does this destruction not take place? Indeed, this has not been known. It seems as if people in the wine-growing districts have paid very little attention to it. The action of tannins has been suggested, and also the acidity, and this destruction has even been denied.

¹ Bouffard et Sémichon: *Compt. rend.*, 126, 423 (1898).

² See *Progrès Agricole et Viticole*, p. 553, 1901, and *Feuille Vinicole de la Gironde*, 1901.

³ *Loc. cit.*

According to our hypothesis, the following must take place in the fermentation-vats: When the fruit is pressed and the cells crushed or destroyed, the enzymic elements which they contain diffuse through the liquid, come in contact, and mutually paralyze one another. What is the mechanism, then, that intervenes in order to eliminate the oxidase and the hydrogenase? It is, in brief, a new force of adhesion that precipitates the mixture of enzymes. Whatever it may be, the external side of the phenomenon is evident.

What, then, is the origin of the reductase that comes into play? It is double. There is at first that of the fruit and then that secreted by the yeast. De Rey-Pailhade has shown that yeast cells are exceedingly rich in hydrogenases. On the other hand, I have found that, contrary to the statements of van Laer,¹ living yeast, in full growth in an active solution, allows its hydrogenase to diffuse completely.² This diffusion takes place under certain conditions which I have determined in an investigation on the formation of hydrogen sulphide in alcoholic fermentations.³

Besides the destruction of the oxidase by the free hydrogenase, another phenomenon takes place, the rôle of which has not yet been definitely determined, though it is not very doubtful. There exists, in the must, free sulphur coming from the sulphur with which the vines are treated, in contact with which the hydrogenases yield hydrogen sulphide that destroys the oxidases.⁴

There remains to be explained the accidental presence of oxidases that finally cause the breaking-down of wine.

Finally, if the fermentation is bad, little hydrogenase is secreted by the yeast, the normal equilibrium is destroyed, and the conditions are reversed, as shown by de Rey-Pailhade.

Law of Reciprocal Action.

Different authors have shown that the above-mentioned

¹ Congrès des Brasseurs belges, Gand, July, 1901.

² "Les oxydases de la levûre," *L'Oenophile*, April, 1902, p. 121.

³ M-Emm. Pozzi-Racot: "Mécanisme du formation de l'hydrogène sulfuré par fermentation alcoolique." *Compt. rend.*, **134**, 628; *Bull. soc. chim.*, **27**, 692 (1902).

⁴ Some preliminary experiments have led me to believe that the oxydases are destroyed by alkaline sulphides and by hydrogen sulphide.

oxidizing enzymes extracted from potatoes do not oxidize salicylic aldehyde in acid, neutral, or alkaline solution. The reason for this has not been clear. It seems to me that this depends on a natural cause; the existence in the tuber of two special classes of enzymic products that are mutually antagonistic, and whose action is governed by a law of equilibrium not well known. There is, on the one hand, a class of oxidases, and on the other, reductases analogous to jacquemase, which is secreted by *Eurotium Oriza*. I have demonstrated the simultaneous existence of these oxidases and reductases. Kastle and Loevenhart¹ have, besides, proved the existence of the oxidases.

It was interesting to find out in which part of the potato these substances with contrary affinities were localized. This is what I investigated. I have shown, first, that if we apply directly the known reactions of oxidases to a slice of potato, it seems that the oxidases are localized in the cells near the periphery, but a more careful study of the phenomenon shows that in the deeper parts there is a state of equilibrium. I have, accordingly, applied to the tuber a method for the separation of the oxidases and reductases, based on the fact that, in a mixture of these two enzymes, the oxidases are more easily precipitated by absolute alcohol than the reductases. I have thus been able to show that all parts of the tuber are equally rich, apparently, in oxidizing enzymes. Why, then, do not all parts of the tuber have the same oxidizing power? This depends simply on a state of equilibrium established between the oxidases and reductases. This state of equilibrium is seen perfectly in an experiment made by the method of Kastle and Loevenhart.

It seems, then, to be well established that there exists a perfect commensalism between the oxidases and reductases. Both are indispensable. The reductases seem to moderate the action of the oxidases. They hinder this as long as no exterior action destroys the equilibrium, and, as shown by Duclaux, we cannot separate the oxidizing from the reducing action, the oxidases from the deoxidases.

¹ This JOURNAL, 26, 539 (1901).

Action of Philothion on the Metalloids of the Sulphur Family.

Philothion acts towards sulphur as a strong hydrogenizing agent. It is a most remarkable property that attracted the attention of de Rey-Pailhade and led him to the discovery of the hydrogenases. In his thesis for the degree of Doctor of Medicine, in 1885, de Rey-Pailhade showed that sulphur is hydrogenized when introduced into the organism, but his first communication on the mechanism of this action is dated June 11, 1888.¹

Before him, Dumas² had established the fact that, when yeast is ground with sulphur and mixed with a solution of sugar in water, there is an evolution of hydrogen sulphide. At the same time that de Rey-Pailhade made this communication, L. Olivier,³ of Pasteur's laboratory, showed that, contrary to the general belief, and the statements of Winogradski,⁴ the organisms of glairin and baregin do not eliminate the sulphur which they contain in the form of sulphuric acid, but that they hydrogenize it. In this communication Olivier showed that, in these organisms, the sulphur plays a part analogous to that of oxygen, and that it is not oxidized by the cells. This is the theory that de Rey Pailhade was to develop later in connection with the ferments of the organism.

I cannot do better than quote a typical passage from this memoir:⁵ "These experiments suffice to prove that the organisms of baregin and glairin use up their intracellular sulphur without oxidizing it. They produce, at the expense of this metalloid, hydrogen sulphide and ammonium thiocyanate, a sulpho derivative of an isomer of urea. This fact, which is entirely new, seems to assign to sulphur a function of which no example has been known in physiology up to the present. Perhaps this substance can take the place of oxygen in the transformation of albuminoids into amido compounds, and, in a general way, in the combustion of living matter."

¹ J. de Rey-Pailhade: "Sur un corps d'origine organique hydrogénéant le soufre à froid," *Compt. rend.*, 106, 1683 (1888).

² T. B. Dumas: *Ann. chim. phys.*, [5], 3, 92.

³ Louis Olivier: "Expériences physiologiques sur les organismes de la glairine et de la barégine,—Rôle du soufre contenu dans leurs cellules," *Compt. rend.*, 106, 1744 (1888).

⁴ Sergius Winogradski: *Botanische Zeitung*, 45, 555 (1887).

⁵ *Compt. rend.*, 106, 1745.

In a memoir published some time afterward, Olivier returns to this question, and shows clearly that the sulphuraires transform sulphur directly into hydrogen sulphide.¹ Olivier and de Rey-Pailhade worked entirely independently. It seems indisputable that the first suggestion of a hydrogenizing enzyme is due to the latter of these two men.

Olivier took into account only the intracellular sulphur,² and did not make a single attempt to isolate the hydrogenizing ferment which he suspected to be present. There is no doubt that de Rey-Pailhade had attempted this in 1886. In the next paragraph we shall see that the production of hydrogen sulphide by the reduction of sulphates has been known for a long time. The mechanism of the reaction may be established, *à priori*, by taking into consideration what we know of philothion and the experiments of Nastukoff.³

To show how easily sulphur is hydrogenized by the solution of philothion as prepared by de Rey-Pailhade, we may proceed as follows: Place some of the solution of philothion, prepared as above, into an Erlenmeyer flask with powdered sulphur or flowers of sulphur, and shake well. Then place over the mouth of the flask a piece of filter-paper saturated with subacetate of lead. Very soon we see that the part directly in contact with the interior atmosphere turns black from the formation of lead sulphide.

This hydrogenation in the cold is quite unique. It is only to be compared with the action of hydriodic acid on sulphuric acid.⁴ It is easy to determine the quantity of hydrogen sulphide formed by the action of a given volume of the alcoholic extract of yeast upon sulphur. The extract of yeast and the sulphur are placed together in a small flask connected with a series of Durand wash-bottles containing a measured quantity of iodine solution of known strength. By means of an aspira-

¹ Louis Olivier: "Nouvelles expériences physiologiques sur le rôle du soufre chez les sulfuraires," *Compt. rend.*, 106, 1806 (1888).

² *Ann. de l'Institut Pasteur*, 1897.

³ Duclaux: "Traité de microbiologie," Vol. III., p. 179. Nastukoff, "Essai sur le pouvoir réducteur des levûres pures—Moyen de le mesurer," *Compt. rend.*, 121, 535 (1895), admits that the sulphates are reduced during alcoholic fermentation. This is denied by Kulisch: *Weinbau und Weinhandel*, (1894). H. Quantin has proved that copper sulphate can be reduced during the fermentation (*Compt. rend.*, 103, 888).

⁴ Berthelot: *Bull. soc. chim.*, 31, 310.

tor, a current of air or carbon dioxide is drawn through in order to carry over the hydrogen sulphide that is formed. The amount of iodine not reduced by the hydrogen sulphide is determined by a solution of sodium thiosulphate. From this we can deduce the volume or the weight of the hydrogen sulphide.

Naturally the amount of hydrogen sulphide produced depends on a number of circumstances. I have obtained very variable figures, and much more variable ones than those given by de Rey-Pailhade. He found that 100 cc. of the active liquid gave more than 1 cc. of hydrogen sulphide. In an experiment published elsewhere, I found 10 cc. De Rey-Pailhade does not state the duration of his observations. The temperature also plays an important part, for philothion, like all other enzymes, has its optimum temperature.

I have shown in a series of investigations that metalloids related to sulphur—selenium, tellurium, arsenic, and phosphorus—are also capable of being hydrogenized.¹ The hydrogenation of phosphorus in the organism has been announced before by Noé. I do not know what his results are.

Of these experiments I shall only state what has been done to show the addition of hydrogen to selenium. A mixture of 800 cc. of the very active hydrogenizing liquid, containing a large excess of antiseptic (sodium fluoride), and 2 grams of selenium was put into a large Erlenmeyer flask, which was closed with a rubber stopper. It was connected with a series of Durand wash-bottles containing caustic soda. The flask was kept for forty-eight hours in a water-bath heated to about 25°. At the end of this time the gas in the flask is driven over through the wash-bottles by means of a stream of carbon dioxide. Then the liquid in the flask is gradually heated to boiling, the current of carbon dioxide being continued. At the end of the experiment the presence of selenium is sought for in the first wash-bottle by microchemical methods,² the results showing that hydrogen has been added to the selenium. Besides, the odor of the gas in the flask was sufficiently characteristic.

¹ M-Emm. Pozzi-Escot: "Contribution à l'étude du philothion," *Compt. rend.*, **134**, 66; *Bull. soc. chim.*, [3], **127**, 346; *Bull. Soc. Hist. Nat. Toulouse*, p. 42, Feb. 5 (1902).

² M-Emm. Pozzi-Escot: "Analyse microchimique et spectroscopique," p. 78.

Reducing Action of the Reductases.

These reducing reactions are very interesting, and it was by a consideration of similar ones that I discovered jacquemase. Indigo-carmin is decolorized by philothion, and the same is true of an alcoholic tincture of litmus. It requires about fifteen hours, at the ordinary temperature, and even less at 35° , to destroy a slight coloration of indigo-carmin. It must be a reduction, for the decolorized liquid, when shaken in contact with the air, at once regains its original blue color. A half liter of the extract easily reduced 0.5 gram of indigo-carmin in a few hours. With the help of an intermediary substance the reaction can be carried out much more rapidly. A few grams of sulphur are put into a small flask, which is then filled with fresh extract, colored with indigo-carmin, corked, and shaken. The blue tint disappears after a few minutes, but returns as soon as the flask is opened. The phenomenon is easily explained: The indigo-blue, $C_{16}H_{10}N_2O_2$, is changed by the influence of the reducing agents into indigo-white, $C_{16}H_{12}N_2O_2$, which contains two more atoms of hydrogen; thus, the philothion and the sulphur yield hydrogen sulphide which, under the influence of the indigo-blue, loses hydrogen with the simultaneous formation of indigo-white (colorless). There is, at the same time, a deposition of sulphur. But indigo-white is a substance very easily oxidized, even if it remains under the liquid.¹

Action of Alkalies.

What precedes has to do with the natural extract, which is slightly acid. De Rey-Pailhade has studied the influence of alkalies. The alcoholic extract, when made alkaline by caustic soda, absorbs free oxygen and rapidly reduces indigo-carmin. The effect is the same with sodium carbonate. The alkaline extract, exposed to the air, loses in a short time its property of producing hydrogen sulphide in contact with free sulphur. Hence, the effect of alkalies is to render the philothion much more sensitive to the action of free oxygen.

¹ Nietzsche: "Chimie des matières colorantes organiques," p. 369 (1901).

Action upon the Tincture of Gum Guaiacum.

The reductases act upon tincture of guaiacum, and this action is important. It was the subject of a memoir presented by me before the Academy of Sciences.¹ It has been known for a long time that the oxidases directly turn to a blue color the white emulsion obtained by putting a few drops of an alcoholic tincture of gum guaiacum into water,² and that other enzymes cause the same transformation when a few drops of hydrogen peroxide are added to the solution. It had also been noticed that certain substances containing known enzymes, for instance, koji, which contains amylase, do not give any coloration under these conditions, and hence, at first sight they seem not to contain an enzyme, although they decompose hydrogen peroxide. Several hypotheses have been suggested to explain this phenomenon, but they are not at all probable.

I showed, as soon as this fact became known, that it was due to the intervention of a new kind of enzyme possessing the property of reducing tincture of guaiacum.³ We can readily prove this by showing that an extract of yeast, prepared with saccharose, which is very rich in zymase and sucrase, as well as in philothion, does not act on the emulsion of gum guaiacum tincture, even in the presence of hydrogen peroxide, but on introducing a small quantity of philothion into a solution of guaiacum blue, which has been prepared beforehand by means of some mineral or enzymic oxidizer, the guaiacum blue is at once reduced.

These facts are of great importance, for they establish clearly that the reductases cannot be detected by a color reaction with tincture of gum guaiacum, and, on the other hand, that in a mixture of enzymes the tincture of gum guaiacum does not give any indication if reductases are present.⁴

From these facts it follows that a certain number of pub-

¹ M-Emm. Pozzi-Escot: "Sur une importante cause d'erreur dans la recherche des diastases," *Compt. rend.*, 134, 401.

² Bertrand: *Bull. soc. chim.*, [3], 11, 717 (1894).

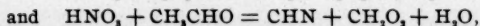
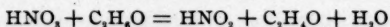
³ A detailed explanation of the phenomenon has been published in a memoir addressed by me to the Academy of Sciences. *Compt. rend.*, 134, 81 (1902); *Bull. soc. chim.*, [3], 27, 280 (1902).

⁴ M-Emm. Pozzi-Escot: *Ann. et Rev. de chim. analyt.*, 7, 185.

lished researches on the enzymes and their localization in special cells, lose their value when we consider that these facts were established by means of colored reagents, upon which all the reductases acted, and that did not take this action into account; and, too, these reductases are very abundant in living things.¹

Action upon Certain Salts Containing Oxygen.

De Rey-Pailhade succeeded in reducing the arsenates to arsenites,² and Rabuteau reduced the iodates and bromates to iodides and bromides, respectively.³ After verifying the work of these authors, I tried to cause the reduction of the nitrates, thinking that this, if successful, would have an interesting bearing upon the mechanism of the assimilation of these salts by plants. We know that, with few exceptions, plants produce their own proteids from mineral nitrogen. Although this has been known for a long time, we do not know through what successive phases or steps ammonia and nitrates pass before being transformed into albuminoid substances, and the mode of formation of the first stage of this transformation, and the nature of the substance which it represents, are still under discussion. It must be remembered that the nitrates enter the plant much dissociated on account of their extreme dilution, and, also, the slight acidity of the plant juices. That is, they are in a particularly unstable condition, the ionic state, which no doubt facilitates the exercise of chemical affinities. Armand Gautier thinks that the nitrates are reduced by the aldehydes in the leaves, according to the equations



or by some similar reaction.⁴ Bach believes that nitric acid is changed by reduction into hydroxylamine.⁵

Schloesing has given an explanation of a different order.⁶

¹ M-Emm. Pozzi-Escot: *Compt. rend.*, 134, 479.

² Rey-Pailhade: *Congrès de Physiologie de Turin*, Sept., 1901.

³ Rabuteau: *Traité de Thérapeutique*.

⁴ Armand Gautier: *Leçons de Chimie biologique*, Ed. 2, p. 36.

⁵ Bach: *Compt. rend.*, 122, 1499.

⁶ Schloesing fils: *Compt. rend.*, 131, 716; *Ann. Agron.*, 28, 144.

The existence of reducing enzymes in living cells, and particularly in growing plants, in the embryo buds at the extremities of the shoots, and in all parts where intercalary growth is not finished (which is just where the nitrates¹ are found) led me to see in the reductases the long-sought reducing agent.

From the coexistence of reductases and nitrates in the same parts of plants, we cannot help drawing conclusions favorable, *à priori*, to this view. In order to determine the exact relation between the nitrates and the reductases, I tried to bring about the reduction of nitrates by means of the reducing secretions of the cells. An active solution of philothion was prepared by one of the methods already given, and the following experiments were made with it :

	Solution of reductase.	Potassium nitrate.
	cc.	Gram.
1	100	0.01
2	100	0.05
3	100	0.10
4	100	0.50
5	100	1.00

At first the reaction of the liquid was slightly acid, indifferent to metaphenylenediamine sulphate, to mercury potassium iodide, and to Trommsdorff's reagent. It did not contain a trace of cyanide.

Each experiment was enclosed in a flask with a ground-glass stopper. At the end of three days the experiment was ended, and the amount of unchanged nitrate determined, as well as the reaction of the liquid. Nos. 4 and 5 were the same as at the start, which is explained by the toxic action of the nitrates upon the hydrogenases, as I have shown elsewhere.²

Nos. 1, 2, and 3 were carefully examined. No. 1 gave freely the reaction for nitrites with metaphenylenediamine and

¹ P. Mazé : "Évolution du carbon et de l'azote dans le monde vivant," p. 84 (Bibliothèque scientifique. Carré et Naud).

² M.-Emm. Pozzi-Escot : "Mémoire sur les actions catalytiques des hydrogénases," *Compt. rend.*, 134, 81; *Bull. soc. chim.*, [3], 27, 284.

It may be added that, in this experiment as in all other similar ones made by me, it has always been shown that a specimen of the diastatic liquid, kept for five minutes at 80° to 90°, remains inactive (neutral) indefinitely.

with Trommsdorff's reagent, and even a slight coloration with mercury potassium iodide. The nitrogen of the nitrate was determined after the destruction of the products of reduction. There was found 0.0041 gram, corresponding to a destruction of 0.0059 gram. When examined in the same way, Nos. 2 and 3 also gave freely the reaction with metaphenylenediamine and with the iodide, and showed, on examination, that some of the nitrogen of the nitrate had disappeared. Must we attribute the observed reduction to the hydrogenizing enzyme? It is not certain, but it seems very probable. When taken in connection with the reduction of the iodates, bromates, and arsenates observed by de Rey-Pailhade, we can be almost sure of it. I wished to carry these experiments still farther, and, in place of extracting the enzyme in order to make it act on the nitrate, I wished to see if it were not possible to obtain a considerable reduction in a cell rich in hydrogenizing enzymes (reductases). For this purpose I used beer yeast that had been selected from the ordinary bottom yeast of the brewery, and grown in a pure culture for some time. This was put into a solution of pure saccharose, which was acidified with nitric acid and contained a suitable quantity of LaClaire's nutrient salts.

The experiments were carried out in Pasteur flasks containing 1 liter of syrup (80 grams of sugar to the liter), slightly acidified with nitric acid, and containing increasing amounts of potassium nitrate as the nitrogenous food, together with 1 gram of the phosphate of the same base.

Of the experiments which were made I will give only two :

- (1) With 4 grams of potassium nitrate, inoculated Jan. 26.
- (2) With 10 grams of potassium nitrate, inoculated Jan. 26.

The fermentation was slow in both flasks. It was hastened by shaking vigorously several times a day at first. The experiment was ended March 15. The yeast was isolated and dried rapidly on filter-paper, and the liquid in the flasks was tested with metaphenylenediamine and mercury potassium iodide. The liquid from flask (1) gave a slight coloration with phenylenediamine sulphate. The liquid in (2) did not react with this substance. Both flasks contained traces of sal

ammoniac. Considering only the liquid in the two flasks, we see that there was a reduction, but in only one case was there found nitrous acid, the intermediate stage.

Nitrates, nitrites, and ammonium salts were then looked for in the yeast cells. After drying the yeast, plasmolysis was brought about by the method already mentioned. In both cases it was shown that the cells contained nitrates, nitrites, and ammonium salts. These experiments seem very conclusive in favor of the reduction of nitrates in plants, although the reducing action of the sugars present is one great objection. In the case considered, which is the reducing agent, the enzyme or the sugar? The enzyme, it seems to me, but it cannot yet be stated positively.¹

Abelous and Gérard found that aqueous extracts, containing chloroform, of certain organs change alkaline nitrates into nitrites.² I did not know of the work of these authors until quite recently, after having made my experiments and written what precedes. If we make a maceration of 40 grams of ground horse kidney in 100 cc. of water, and add 8 grams of potassium nitrate, and some chloroform to prevent the action

¹ I have recently undertaken new experiments, working with the young buds of trees. ("Étude sur le mécanisme de la réduction des nitrates chez les végétaux; intervention d'une action diastasique," *Compt. rend.*, 134, 863.) I have thus been able to show clearly that there is really a reduction of nitrates under the influence of reducing enzymes. In the phenomenon of assimilation their intervention is undeniable. Experiments along this line were recently made with stems of burdock gathered at different times of the day. They were ground in a Simon grinder, then in a mortar, and finally squeezed in a press. The liquid was set aside. The cake was put into water, washed, and pressed, and the new liquid put with the first. An excess of ammonium fluoride was added and the whole divided into three equal parts, A, B, and C. A was let alone. B was heated to 85° to 90° for five minutes. To C was added nitrate of potassium. The three were left in a water-bath heated to 35° for fifteen to eighteen hours, at the end of which time it was found that a part of the nitrates existing naturally in A had been reduced to nitrites and ammonia derivatives decomposable by boiling with caustic soda. In C the action had been the same. In B the liquid remained as it was at first. In A and in C the ammonia was determined by treatment with caustic soda, figures much higher than for B being found. There had been, then, reduction and diastatic assimilation. These experiments have shown that the enzymes that reduce nitrates are very abundant, at the end of warm days, in actively growing plants, but that there is very little for some hours after sunrise, and after a cool night. From my experiments I believe that there is a difference between philothion, jacquemase, and the enzyme that causes the reduction of nitrates. I suggest for this the name of jacquemase β , jacquemase α being the first one discovered by me.

² Abelous et Gérard: "Sur la présence dans l'organisme animal d'un ferment soluble réduisant les nitrates," *Compt. rend.*, 129, 56.

of micro-organisms, we find that, after being warmed to 40° for twelve to fifteen hours, the filtrate shows that the nitrate has been reduced. If we work in the same way with ground kidney, which has previously been heated to 100°, there is no reduction. If we examine, under the same conditions, the reducing power of the different organs of the horse, it will be found that most of them reduce potassium nitrate, though not all to the same extent. The precautions taken by these authors do not allow us to attribute the reactions considered to the intervention of parasitic micro-organisms. Finally, from the systematic way in which they studied the influence of temperature upon the progress of the reduction, there seems to be no doubt that in this case the action is due to enzymes.

Abelous and Gérard afterwards made a comparative study of the reducing power of different tissues. The following is quoted to show their method of work :

"1. Two hundred and fifty grams of ground kidney are macerated in 300 cc. of distilled water, with the addition of chloroform, for twenty-four hours at 42°, and then filtered.

"(a) To 100 cc. of the limpid filtrate are added 8 grams of potassium nitrate and 1 cc. of chloroform.

"(b) One hundred cc. of the filtrate are heated to boiling, then 8 grams of potassium nitrate and 1 cc. of chloroform are added.

"After allowing them to stand four hours in an air-bath at 40°, the presence of nitrites is tested for by means of zinc iodide, starch, and acetic acid (Tromsdorff's reagent). It is found that (a) gives a decided reaction, while (b) gives none.

"The mixtures are left twelve hours longer, at the end of which time (b) gives no reaction, while with (a) the reaction is more decided than before. Hence an aqueous maceration of horse kidney is capable of reducing nitrates. The temperature of 100° destroys this property."¹

They have also shown that the ferment² of this reduction is soluble in glycerin. These experiments confirm what has preceded.

¹ Abelous and Gérard: "Sur la présence dans l'organisme animal d'un ferment soluble réducteur—Pouvoir réducteur des extraits d'organes," *Compt. rend.*, 129, 164.

² I revive here the word "ferment" which should be understood as "chemical agent."

Abelous and Gérard published a second note,¹ which, I believe, should be sharply criticized. They admit that the reduction of the nitrates into nitrites is counterbalanced, in the living tissues, by the opposite action brought about by the intervention of the oxidases. In my opinion, this is self-contradictory, and is entirely wrong, for it is impossible not to see in the reduction of nitrates to nitrites the first stage of a complex mechanism, known in ordinary language by the name assimilation.

The Hydrogenation of Nitrobenzene.

Letheby² stated that nitrobenzene is reduced and transformed into aniline in the body. Abelous and Gérard,³ in repeating this work, were led to interesting results. They extracted from horse kidney a reducing enzyme, and showed its action upon nitrobenzene. Ground horse kidney is macerated with its own weight of distilled water for twenty-four hours at 42°, in an atmosphere of hydrogen, some chloroform being added. It is then filtered.

(a) To 100 cc. of the limpid filtrate are added 40 drops of nitrobenzene and 2 cc. of chloroform, and the whole is then enclosed in an atmosphere of hydrogen.

(b) To 100 cc. of the same filtrate, brought to boiling, are added the same quantities of nitrobenzene and chloroform in a flask filled with hydrogen.

The two flasks, inverted under water, are placed for forty-eight hours in an air-bath heated to 42°. At the end of this time each is shaken with ether. The latter is then decanted, evaporated, and the residue tested for aniline by treating it with a solution of calcium hypochlorite. With (a) there is obtained a fine bluish-violet coloration, passing gradually into red. On the contrary, (b) gives no color.

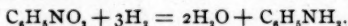
Hence, a clear, aqueous extract of horse kidney, containing chloroform, is able to transform nitrobenzene into anil-

¹ Abelous et Gérard: "Sur la coexistence d'une diastase réductrice et d'une diastase oxydante dans les organes animaux," *Compt. rend.*, 139, 1023.

² Würtz: "Dictionnaire," 1^{er} Supp., Vol. I., p. 284.

³ Abelous et Gérard: "Transformation de la nitrobenzine en phénylamine par un ferment réducteur et hydrogénante de l'organisme," *Compt. rend.*, 130, 420 (1900).

ine by deoxidation and hydrogenation, according to the following equation :



The reaction is due to the action of an enzyme, as shown by the influence of heat. Although Abelous and Gérard knew about philothion, discovered previously by de Rey-Pailhade in the kidney of the horse, they did not mention this hydrogenizing ferment in their memoir, and did not think of attributing to its action the new phenomena which they made known. De Rey-Pailhade replied to this injustice by a brochure, "*Le philothion ou hydrogénase*,"¹ in which he tried to establish by thermochemical considerations that philothion is able to cause the hydrogenation of nitrobenzene. I have tried to verify the fact directly by experiment.

Using a solution of philothion, I repeated the experiments of Abelous and Gérard, and obtained the complete reduction of nitrobenzene. It is an easy experiment to carry out. Five hundred grams of compressed yeast are treated with a convenient plasmolyzing agent, a sufficient amount of ammonium fluoride is added, and then a few cubic centimeters of nitrobenzene. The whole is then placed in a flask in a water-bath at 35°. At the end of forty-eight hours it is treated with ether. The ether is decanted, evaporated on the water-bath, and aniline is tested for by the usual reagents, especially that of Jacquemin. There seems, then, to be complete identity between the ferment of Abelous and Gérard and philothion. I might remark besides, that there is no doubt about the true nature of the ferment discovered by Abelous and Gérard, and on the presentation of one of my notes to the Academy of Medicine, Armand Gautier identified it with philothion.²

Reduction of Sulphates.

It seems certain, at the present time; that sulphates are reduced during fermentation, as was shown by Quantin in connection with sulphate of copper,³ and Nastukoff showed the

¹ "*Le philothion ou hydrogénase: Considérations sur les expériences de MM. Abelous et Gérard*," Toulouse (1900).

² Armand Gautier: *Bull. Acad. Med.*, [3], 47, 400 (1902).

³ H. Quantin: "*Sur la réduction du sulfate de cuivre pendant la fermentation du vin*," *Compt. rend.*, 103, 888.

same for sulphate of magnesium.¹ Finally, it appears from different authors that the reducing agent is philothion, or a closely related reducing enzyme. It has been shown that in industrial fermentations there is very often a formation of hydrogen sulphide, even in a pure fermentation.² It is due to the reduction of the sulphates existing in the must, and is evidently caused by an enzyme.

Another fact which seems to speak in favor of the reduction of sulphates by reductases has been studied by Olivier;³ that is, the production of hydrogen sulphide by sulphuraires.⁴ It was shown long ago that there always exist bacterial organisms in all sulphur springs.⁵

These organisms are of especial interest, for nearly all of their cells enclose granules of sulphur. This shows that they exercise a certain influence upon the composition of the water—they reduce the sulphates. Olivier⁶ proved later that the sulphuraires transform sulphur directly into hydrogen sulphide. His experiments upon this are very remarkable. He takes fresh, well-washed baregin, puts it in a flask with barium chloride, and shows that hydrogen sulphide, mixed with carbon dioxide, is given off. According to him, sulphur is changed into hydrogen sulphide, under the influence of some unknown enzyme, without leaving the organism as

¹ Nastukoff: "Pouvoir réducteur des levûres pures: moyen de le mesurer. *Compt. rend.*, 121, 535 (1895).

² The fact was recently discussed before the Association de Sucrierie et de Distillerie in a communication by Emile Barbet regarding my investigations. (June 2, 1902.)

³ L. Olivier: *Bull. Soc. Bot. France*, p. 29 (1882); A. Etard et Olivier: *Compt. rend.*, 93, 846 (1882); L. Olivier: "Sur la flore microscopique des eaux sulfureuses."

⁴ See Duclaux: *Traité de Microbiologie*, 2, 177. Duclaux seems to believe that philothion exists only in yeast cultures in suitable media. This is incorrect, for there are always reducing enzymes in all yeast cells, no matter how they are grown. The latter influences only the mass of the cells.

⁵ The organisms are rare even in the spring itself. They are small, motile rods, very transparent and difficult to observe. The farther we go from the source, and as the water cools, it is found that the rods or filaments multiply, and at about 50° there appear the masses of baregin that are nothing but a zooglyca of rods mixed with grains of reduced sulphur upon which are developed sulphuraires similar to the filaments in the spring, but more elongated and enclosing characteristic granules of sulphur. See A. Certes et Garrigon: "De la présence constante de micro-organismes dans les eaux de Luchon, recueillis au griffon à la température de 64° et leur action sur la production de la barégine." *Compt. rend.*, 103, 703 (1886).

⁶ L. Olivier: "Nouvelles expériences physiologiques sur le rôle du soufre chez les sulfuraires." *Compt. rend.*, 106, 1806 (1888).

sulphate, and the decomposition of the hydrogen sulphide by the humidity and the oxygen of the air is the cause of the precipitation of free sulphur.

Olivier has shown that there are formed by the action of glairin and of baregin, equal volumes of carbon dioxide and of hydrogen sulphide, which leads him to suppose that the sulphur bacteria assimilate at the same time sulphur and oxygen, at the expense of the alkaline sulphates, thus producing carbon oxysulphide instead of carbon dioxide. This gas then combines with water, regenerating carbon dioxide and hydrogen sulphide.¹

All these facts lead to the conclusion that the sulphates are reduced by secretions related to philothion, and evidently acting like enzymes and hydrogenizing reducing agents. It is a matter of scientific interest to clear up this point.²

There exists a whole class of bacteria, the *Bacilli sulfhydro-geni* of Miquel,³ which have the property of liberating carbon dioxide and hydrogen sulphide when grown in a medium containing either free or combined sulphur. Besides, the bacteria that can give hydrogen sulphide with free sulphur are very numerous,⁴ so that this property is not to be regarded as a specific character. To show this, it is only necessary to suspend in the culture-flasks strips of paper saturated with lead acetate.⁵ They frequently turn black. On adding washed flowers of sulphur to the cultures the phenomenon becomes much more marked, and streams of hydrogen sulphide are liberated.

We do not know the mechanism of this hydrogenation, but

¹ Than: Ann. Chem. (Liebig), Suppl., 5, 236.

² M-Emm. Pozzi-Escot: "Mémoire sur les propriétés catalytiques des hydrogénases," Compt. rend., 134, 81.

³ Miquel: "Sur la fermentation sulfhydrique," Bull. soc. chim., [2], 32, 127; "Biogénèse de l'hydrogène sulfuré par les bactéries," Ann. de Micro., 1880.

⁴ Holschenikoff: "Sur la formation de l'hydrogène sulfuré par les bactéries," Ann. de Micro., 1880.

⁵ Rosenheim: Soc. de médecine interne de Berlin, 1887. This author has found, in urine containing hydrogen sulphide, bacteria that gave off this gas when in contact with free sulphur, and concluded, wrongly, that the odor of hydrogen sulphide detected in the urine at the moment of its emission was the result of bacterial action. We now know that the enzymes of the kidneys, which may diffuse into the urine under certain conditions, give hydrogen sulphide when in contact with sulphur or sulphates, and that the bacteria are able to cause the same phenomenon on their own account.

believe it is due to the formation of nascent hydrogen. It would be interesting to see whether these organisms are not rich in reducing enzymes, as seems probable. I have not had time to verify this fact, which appears to be true, *à priori*. They have been introduced into science under the name "sulphur bacteria,"¹ since they can oxidize hydrogen sulphide and thus form deposits of sulphur.

Winogradski² maintains that this oxidation can proceed as far as sulphuric acid. This point is still very obscure. There is some relation, not yet evident, and a mechanism very interesting to study, between the sulphuraires and the sulphur bacteria.

*Action of Reductases upon Hydrogen Peroxide.*³

It has been known for a long time that enzymes, in general, decompose hydrogen peroxide, and it is customary to characterize the action of an enzyme by the oxidation of an emulsion of an alcoholic tincture of gum guaiacum, in the presence of hydrogen peroxide. The latter, under the influence of the enzyme, gives the oxygen necessary to transform the guaiaconic acid into guaiacum blue. I was the first to show that the action of the reducing enzymes is quite different, yet the hydrogen peroxide is decomposed, and there is a very active liberation of oxygen, amounting, with an excess of peroxide, to several hundred cubic centimeters in a few minutes. The enzyme seems, at first sight, to act like a substance having the properties of a catalytic agent.

It is interesting to note that although philothion decomposes hydrogen peroxide, it does not give the characteristic reaction with gum guaiacum in the presence of the peroxide.

¹ Petri and Maassen: Arb. aus dem Kaiserl. Gesundheitsamte, 8, 318 (1893); Morris: Arch. für Hygiene, 30, 304 (1897).

² Winogradski: Botan. Zeitung, Nrs. 31-37 (1887); E. Wollny: "La décomposition des matières organiques et les formes d'humus dans leurs rapports avec l'agriculture (Trad. de E. Henry)", p. 53.

³ M-Emm. Pozzi-Escot: "Mémoire sur les propriétés catalytiques des hydrogénases," Compt. rend., 134, 81. "Propriétés catalytiques des hydrogénases; identification de la catalase de Loew et du philothion de de Rey-Pailhade," Bull. soc. chim. [3], 28, 280, 288; "Sur un élément diastasique nouveau de l'urine," Compt. rend., 134, 134; Ann. et. Rev. de Chim. analyt., 7, 212; Ann. Soc. roy. méd. Gand; "Mémoire sur les principes réducteurs des tissus vivants et leur identification avec la catalase de O. Loew."

It is well to recall that catalyzers give this reaction. Manganese dioxide gives it even in the absence of hydrogen peroxide. We may explain this anomaly by supposing that the nascent oxygen is insufficient to cause the reaction, and that there must be the intervention of another oxidizer,¹ or else that the guaiacum blue tends to be formed, but is immediately destroyed by the reducing enzyme, which would be still another proof in favor of its existence. Several facts lead us to the conclusion that the latter is the only good explanation, especially the decomposition of the previously formed guaiacum blue by the action of philothion.

In trying to discover the law of the decomposition of hydrogen peroxide, we were led to measure the amounts of oxygen set free by the same quantity of enzyme with increase of time, and to determine the influence upon this action of the substances which Duclaux calls paralyzers. The solution of enzyme that was used was prepared in the way we have already described in this article. When shaken with an excess of a twelve-volume solution of medicinal hydrogen peroxide, 10 cc. of such a solution gives, in eight minutes, 182 cc. of oxygen measured at 15° and 760 mm. We have, besides, followed the general course of the liberation throughout the experiment, with the following interesting result :

Liberation of Pure Oxygen by 10 cc., of the Solution of Hydrogenase Acting upon an Excess of Hydrogen Peroxide.

Time in seconds.	Volume in cc.	Time in seconds.	Volume in cc.
0	0	135	99
25	15	165	111
30	30	180	120
45	50	240	138
60	68	300	149
75	77	360	160
90	82	420	169
105	87	480	178
120	93	540	183.5

To make the liberation of gas more regular, the liquid was shaken almost constantly, and always before each reading. If a mechanical agitator had been used it is probable that

¹ We owe this hypothesis to Oscar Loew.

there would not have been any irregularities in the table. The general course of the phenomenon is interesting. At first the action is very rapid, then it gradually becomes slower, and finally, at the end of a variable time, the enzyme becomes absolutely inactive. In the following table the liberation of gas from minute to minute is given :

Time. Minutes.	Gas set free. cc.
1	68
2	25
3	27
4	18
5	11
6	11
7	9
8	9
9	6.5

In what way does philothion cause the decomposition of hydrogen peroxide? Does it act as a catalyzer, or does it attack the hydrogen peroxide directly, setting free a molecule of oxygen upon which its labile hydrogen acts, giving water and gaseous oxygen, according to an equation analogous to Hoppe-Seyler's? It is not easy to decide between these two hypotheses. It seems more probable that there is no catalytic action, but rather a more or less complex chemical action, as we must suppose from the action of the paralyzers. It is, without doubt, the oxidation of the hydrogenase that causes the reaction to become slower.

It is worth mentioning that the solution of hydrogenase obtained from beer yeast, by means of alcohol, has an acid reaction. Its acidity, calculated in terms of sulphuric acid, is 1.200 grams.

We have shown experimentally that, after acting forty minutes upon hydrogen peroxide, its catalytic power becomes zero. We have also studied the influence of temperature on the reaction. Our experiments were carried out at 15°, and we have shown that the action is greatest between 30° and 40°. De Rey-Pailhade gave the latter temperature as the optimum for philothion. But we have found that if, at this tempera-

ture, the liberation of oxygen was more active at the beginning, there was a marked difference in the curve showing the rate of the action. At 40° the activity entirely ceases in ten minutes; at 60° the action lasts but a few minutes; and above 70° a large coagulum is formed, and the enzyme entirely loses its activity.

The action of paralyzers is of great interest. In the following table is shown the time necessary to obtain 40 cc. of oxygen with 10 cc. of philothion, 0.5 gram of each of the salts, and an excess of hydrogen peroxide:

Paralyzer.	Time in minutes and seconds.	
	Minutes.	Seconds.
Blank	0	45
HgCl ₂	infinite	
AgNO ₃	"	
LiCl	3	15
MnCl ₂	5	3
CaCl ₂	3	25
NaCl	5	8
KCl	5	12
H ₂ KPO ₄	4	54
KNO ₃	11	20

In a general way, the most energetic paralyzers are the salts with an acid reaction; then come the nitrates. Mercuric chloride and silver nitrate have a very powerful depressive action. A solution giving, in a blank experiment, 180 cc. of oxygen in nine minutes gave, in the case of mercuric chloride, only 5 cc. of oxygen in four minutes, at the end of which time the action had apparently ceased. With silver nitrate 3 cc. were obtained in five minutes. In large excess, chloroform has a noticeable paralyzing action. The following shows the course of the experiment:

Time in seconds.	Oxygen set free. cc.
30	18
60	23
120	26
180	33
240	39
300	45

A blank experiment gave 180 cc. of oxygen in nine minutes. An experiment was carried out with a large excess of chloroform (see table on page 546).

The following results were obtained with ferrocyanide :

Time in seconds.	Oxygen set free. cc.
30	25
60	35
90	50
120	60
150	70
180	75
210	78
240	81

The following figures were obtained with calcium chloride.

Time in seconds.	Oxygen set free. cc.
15	12
30	17
45	18.5
60	20
120	22
180	39
240	51
300	58
360	62

As I have already stated, the solution of the enzyme which was used in these experiments had an acid reaction. However, the addition of any considerable amount of a mineral acid or a strong organic acid, such as acetic acid, acts as a strong paralyzer. On the contrary, traces of alkali are very active, provided the alkalinity is very slight. The following experiment shows this :

A blank gave, in one minute, 25 cc. of oxygen.

With 0.5 cc. of acetic acid there were set free 12 cc. of oxygen.

With 0.5 cc. of ammonium carbonate there were set free 38 cc. of oxygen.

In the preceding experiments it would have been better to have worked with neutral solutions, but the results are com-

parable, nevertheless, for all the solutions had the same initial acidity.

The power of decomposing hydrogen peroxide to the extent seen in the case of philothion is not possessed by any other enzyme, to my knowledge, except by one of a new order, just made known by Oscar Loew, that I have studied. The commercial enzyme, taka-diastrase, which is a commercial article extracted from *Aspergillus Oriza*, has the same catalytic action towards hydrogen peroxide. But it is a mixture of enzymes that have not yet been well characterized and in which I have recently discovered the reductase, jacquemase.

The enzyme discovered by Loew, and named catalase by him,¹ is characterized by its catalytic action towards hydrogen peroxide. It is found in vegetable organs. Struck by the close resemblance of philothion and catalase in their action on hydrogen peroxide, alone and in the presence of paralyzers, I thought it well to study this enzyme and compare it with philothion.

A solution of catalase was prepared according to the directions of Loew,² as follows: We obtained several leaves of tobacco, dried in the open air, and some tobacco stems, also dried at the ordinary temperature, from a Belgian plantation. After being finely chopped, 500 grams were digested in 200 cc. of water containing chloroform. The extract thus obtained gave a precipitate of the enzyme when saturated with ammonium sulphate. A second solution was prepared from magnolia leaves. In this case the enzyme was not precipitated but the solution was used at once.

The catalytic enzyme thus obtained corresponded to the form β of Loew. There is no real difference between the form α and the form β of Loew, and the distinction established by him has no scientific value.³ An experiment was made in order to determine the catalyzing power of the en-

¹ M-Emm. Pozzi-Escot: "Sur une nouvelle diastase," Rev. gén. de chim., 5, 182, 194.

² Oscar Loew: U. S. Dept. Agr. Rep., No. 68, p. 39 (1901).

³ Loew made a hypothetical distinction without great value, in my opinion. The soluble form of catalase was called β , and the insoluble form α ; that is, one form can be extracted with water and the other cannot. He seems not to understand the property of adherence which is peculiar to the enzymes.

zyme thus obtained. To 10 cc. of the active solution was added an excess of hydrogen peroxide, and the oxygen set free was measured as in the experiments with philothion. There were obtained the following results :

Time. Minutes.	Oxygen. cc.
1	15
2	22
3	26
4	28.4
5	30
10	33.4
15	35
20	36.7
25	38

The figures are lower than those obtained with the extract of beer yeast, but they are of the same order as those cited by Loew.

An experiment carried out to see whether the enzyme obtained by Loew, and which we prepared for our work, was able to hydrogenize sulphur, gave a positive result. So far there is no difference between the general properties of Loew's catalase and philothion. One point, however, remained to be cleared up. Loew described catalase as an oxidase. Would it be possible to obtain oxidation by means of philothion? *A priori*, it did not seem impossible, for every reduction is the correlative of an oxidation. Besides, we often find catalytic properties and oxidizing power combined, well-known instances being platinum black and manganese peroxide. Platinum black can also cause reduction, for it reduces ammonium nitrate in the presence of glucose which is, at the same time, oxidized. Hence, the idea of an enzyme having both reducing and oxidizing power is not a new one. Philothion and catalase are without action on an alcoholic tincture of gum guaiacum, but that is not sufficient evidence. We have also tried all the known reactions of enzymes, but without result. The reaction with tetramethylparaphenylenediamine failed, and the reactions with indophenols and tyrosine indicated nothing. This does not mean that a reducing en-

zyme cannot yield oxidation-products. These have been noticed, but they are the result of a secondary reaction. Our results only show that, towards these reagents, philothion and catalase do not act in the same way.

Loew claims to have oxidized hydroquinone,¹ which I did not succeed in doing, no matter what means were employed. The hydroquinone was oxidized neither by Loew's enzyme nor by a very active solution of philothion.

It should be stated that Loew found that catalase, when of animal origin, caused perceptible oxidation, but none at all when it was obtained from plants. An oxidizing agent might also have been concerned in the experiments of Loew. However, from his results, it seems to me that catalase is a reductase and is identical with philothion. If we consider the results obtained in the decomposition of hydrogen peroxide by philothion and by catalase, one is struck by their close agreement, which would not be the case with two different enzymes. It has also been shown that the optimum temperature is practically the same for each of them, which is a criterion of the first order. Both give hydrogen sulphide with free sulphur, both have strikingly similar reducing action, and both are found in the same organs. It seems, then, justifiable to conclude that Loew's catalase is identical with the enzymes of the new group which I have established. In my article on the subject² is given all that is known of catalase.

Catalytic Properties of Fibrin.³

The catalytic properties of fibrin, observed by me, led me to investigate whether the decomposition of hydrogen peroxide by fresh fibrin was not a reaction of the same order. We know with what ease the enzyme complexes attach themselves to certain substances.

Fibrin was first made from large quantities of blood, taking all the precautions recommended in the memoirs on the subject. It was shown that the very active fibrin thus obtained

¹ Oscar Loew: U. S. Dept. Agr. Rep., No. 68, p. 39 (1901).

² M.-Emm. Pozzi-Escot: "Sur une nouvelle diastase," *Rev. gén. de chim.*, 8, 182, 194.

³ M.-Emm. Pozzi-Escot: "Sur les hydrogénases du sang et les propriétés catalytiques de la fibrine," *Compt. rend.*, 134, 446; *Bull. de l'Acad. de Médecine*, March 25, 1902; *Bull. soc. chim.* [3], 27, 449; *Ann. soc. Méd. de Gand*, July, 1902.

yields to suitable solvents a substance with catalytic and reducing properties. Hence fibrin is the vehicle, in the blood, of the reducing enzymes that it protects, and the activity of which it moderates more or less completely. When extracted from the blood, the fibrin carries with it the greater part of the reductases.¹

Action of Physical Agents.

Heat accelerates the action of the reductases, up to 45°. This seems to be the optimum for philothion, as well as for jacquemase. At about 60° the activity of the enzyme seems suspended. Loew maintains, nevertheless, that the enzyme that I have identified with the reductases resisted the action of boiling absolute alcohol (78°) for twenty seconds. I have not had an opportunity to verify this fact.

My experiments on the action of light are not yet completed. It seems to me that the reductases obey the general law, and decompose much more rapidly in direct sunlight than in a diffused light or in darkness.

I have tried to study the action of electricity,² but it is very difficult to distinguish the effect of the electricity from the heat produced by the passage of the current, according to the law of Joule, and also to avoid the action of the salts used to carry the current. In the vessel used for the electrolysis we may isolate the electrodes, and consider the intermediate part, alone, as acted on by the current, but we know that the ions move throughout the entire liquid, and there is nothing to show that they do not act under these conditions. A long-continued current, if relatively intense and passed long enough, destroys the action of the enzyme.

The Reductases as Oxidizing Agents.

The internal mechanism of the possible action of the reducing enzymes is a subject of interest. My own investigations on the subject are hardly begun, and I can only mention a few facts here and there for lack of time.

¹ M-Emm. Pozzi-Escot: "Sur les principes réducteurs des tissus vivants, et les propriétés catalytiques de la fibrine," Bull. général de thérapeutique, 142, 764.

² M-Emm. Pozzi-Escot: "Action du courant électrique sur les hydrogénases," Compt. rend., 134, 678.

The following quotation from the note by which Armand Gautier introduced two of my papers before the Academy of Medicine, will serve to introduce this paragraph.¹ His explanation will make clear the great importance of the problem: "A young and very active scientist of Nancy, M-Emm. Pozzi-Escot, asks me to present to the Academy two interesting memoirs entitled, 'Catalytic Properties of Hydrogenases,' and 'Contribution to the Study of the Reducing Substances in Living Tissues.' The author recalls that in 1888 de Rey-Pailhade discovered in living tissues, in yeast and, in general, in young cells, a substance which could cause reduction, that he called philothion on account of the property it possessed of liberating hydrogen sulphide in the presence of free sulphur. He found later that this substance acts like a hydrogenizing ferment. It was, in fact, a hydrogenase.

"Starting from these remarkable observations, Pozzi-Escot studied the part played by this hydrogenizing ferment, and concluded, especially from his work on beer yeast, in which the ferment is quite abundant, that it has the double property, on the one hand, of reducing directly or of hydrogenizing organic substances in the tissues, and on the other, of oxidizing them by a reaction indirectly foreseen by Hoppe-Seyler, which consisted in decomposing a molecule of free oxygen or that which is so lightly held by an inactive molecule of haemoglobin that it is still composed of 2 atoms. But in the presence of the hydrogenase, one of these atoms unites with hydrogen from the ferment to form water, while the other is set free in the nascent state or as an ion, and has active oxidizing power.

"Pozzi-Escot identified his hydrogenase with philothion, and with catalase, obtained by Loew from tobacco leaves and which he believed to be an oxidizing ferment, and showed that it acted like a true hydrogenase. Finally, he showed that the hydrogenase exists in the blood, and that it combines with the fibrin as it coagulates.

"The existence of hydrogenases in living tissues has been confirmed, besides, by Gérard and Abelous for the kidney, a

¹ Armand Gautier: *Bull. Acad. Médecine, Paris*, [3], 47, 400 (1902).

fact that makes of still greater importance the views I expressed long ago concerning the internal mechanism of the reactions taking place in the protoplasm of the living cell. The reactions that take place in its depths, far from being phenomena of oxidation, as has been thought since the time of Lavoisier, are phenomena of hydrolysis and of reduction. The oxidations do not take place until later, and only in the external or superficial parts of the cell, and at the expense, not of the protoplasm itself, but of its decomposition-products formed by the first stage of the vital function."

We know, in a general way, that certain oxidizing or reducing processes are reversible under certain conditions. We need only mention the oxidizing power of platinum-black and its reducing action towards nitrates, which it transforms into ammonia under certain conditions.¹

The process of oxidation in the blood has been studied by many authors, all of whom have had in mind the action of an oxidase. It has been found that a pulp made from the organs of different animals, or even the extract prepared from them, can oxidize benzoic aldehyde, salicylic aldehyde, and formic aldehyde, transforming them into the corresponding acids. Methyl alcohol can be oxidized, but less readily, into formic acid, benzyl alcohol into benzoic acid, and even the oxidation of acetone and uric acid has been observed.

Spitzer also attributes to the action of oxidases certain color reactions, such as the formation of indophenols, of Bindschedler's green, and of toluene-blue. Binz showed that animal extracts can transform arsenious acid into arsenic acid. These so-called "oxidasic" oxidations are generally of a singular nature. Pohl prepared two extracts that would oxidize aldehydes but not give the indophenol reaction. Jacobs decomposed uric acid by means of dog's liver, but not with that of a calf. Spitzer also found that glucose is oxidized by the blood and by various extracts of animal tissues. The enzyme that caused these oxidations was called the glycolytic ferment and Spitzer said that it gave a blue color with an alcoholic tincture of gum guaiacum, and had, at the same time, a catalytic action on hydrogen peroxide.

¹ Ber. d. chem. Ges., 23, 675 (1890).

Jacobs, although he cast a doubt upon this view, admits the existence of several oxidizing enzymes in animal tissues, and believes, also, that the enzyme that causes the oxidation of sugar is different from that which transforms benzoic aldehyde into benzoic acid.¹

It must be remembered that, in many cases, the action of the enzymes is specific; that is to say, they act on certain classes of substances containing groupings, the reactivity and even the configuration of which corresponds to that of the enzyme.²

If, as we believe, the reductases are susceptible of producing oxidations and reductions at the same time, this would take place only with substances upon which the enzyme could not act as a reducing agent without rapidly reaching a state of equilibrium, a limit closely dependent upon the conditions of the reaction.

This is what takes place usually. If we wish to show clearly the secondary oxidations (see the definition of Armand Gautier), it is absolutely necessary to employ certain experimental methods that remove as far as possible the limit of the reaction.

We have seen that oxidations by means of oxidases are possible only at the exterior of the cell or of the tissues and that, whenever internal oxidation takes place, it is due to a secondary action closely dependent, in some way difficult to conceive, upon a reducing enzyme.

I was led to the observation of these remarkable phenomena by an investigation carried out to see whether oxidizing enzymes exist in the cells of beer yeast. It has been stated that oxidases exist in this organism, but no real proof of it could be cited. It was only because, from its superficial properties, it acts like an agent of combustion. Duclaux stated that some oxidase exists in Buchner's press-juice.

The subject is still very perplexing, for the yeast acts like a plant. It respires, and the respiratory quotient, $\frac{\text{CO}_2}{\text{O}}$, may have, according to the vital condition, a value varying from

¹ Jacobs : *Virchow Archiv.*, 187, 235 (1899).

² This last condition is given with all due reservation.

unity to infinity. At first sight the yeast cell seems to be aerobic, on account of the secretion of oxidases. This was the leading idea in the investigations of Effront and Grüss, which are, however, inexact. I sought these oxidases at first in the extract of yeast, but this is such a reducing agent that one is bound to fail in trying to detect any oxidizing action. One must not be deceived and admit certain experiments as exact, for the reductases have very great vitality, much greater than the oxidases. This is, I think, an objection to certain of the experiments of Grüss, which I criticized when they were published,¹ and to which I will not return.

I have also tried to detect the presence of oxidizing secretions by working with actively growing yeast, but with complete lack of success, for in this condition, also, the yeast causes reduction, and takes up oxygen readily. On the one hand, the cell-sap of the yeast is essentially a reducing agent, and under no circumstances can it produce oxidation.² On the other hand, the living cell causes reduction, and absorbs the oxygen, which it needs to continue its growth, from the oxygen compounds around it.

Grüss and Effront had, nevertheless, characterized certain oxidizing secretions of the yeast cell. I think I have shown this to be wrong, but it led me to see whether the reductases might not, under certain conditions, act as indirect oxidizing agents. With this end in view, I made the following experiments:

1. Five hundred cc. of a very active solution of de Rey-Pailhade's hydrogenase, prepared as already described, were put into a 750 cc. flask, then 1 gram of salicylic aldehyde was added. The flask, incompletely filled, was closed and so shaken as to bring all of the liquid in contact with the oxygen it contained. At the end of several hours the liquid was made alkaline with sodium carbonate and evaporated to dryness in an air-bath at 105°. The residue was washed with 200 cc. of 90 per cent alcohol, in several portions, and the solution obtained evaporated to a small volume. The residue

¹ "Sur les oxydases de la levûre," *Compt. rend.*, 134, 377; *Revue Oenophile*, 9, 118.

² It all depends on the manner of life of the cells studied. From this point of view there are very great differences between them.

was acidified with hydrochloric acid and extracted with ether. The ethereal solution, when poured off and evaporated, yielded a small amount of salicylic acid. It is a question whether this oxidation is the result of a primary or secondary reduction by the reductase.

2. Experiments were made in all respects similar to the one above, but to the solution was added some easily reducible substance, such as an iodate, an arsenate, etc. Under these conditions, and working in the same way, the amount of salicylic acid formed was increased. From this we must conclude that the oxidation was due to a secondary action accompanying the true reduction.

3. Several other series of experiments were made in which benzyl alcohol was the substance oxidized. The results were conclusive, for in no case was any benzoic acid formed.

These experiments are not satisfactory, but they are interesting, and for that reason have been mentioned.¹

Physiological Rôle of the Reductases.

Reducing agents are as important in the organism as oxidizing agents, and the importance of the reductases is also considerable.² The phenomenon of disassimilation is intimately connected with these secretions. De Rey-Pailhade wished to treat the subject in the preface to my work, but I only mentioned the occurrence of these enzymes in the urine.³ They come from the kidneys, and can pass into the urine only as the result of a physiological accident. This has been brought to the attention of physiologists.

From another point of view the reductases play an important part in nature. I have shown that they decompose hydrogen peroxide, giving free oxygen. Also the investigations of De Rey-Pailhade and myself have shown that they are very abundant in plants and animals. There must be some reason

¹ I showed recently that yeast contains spermine, thus demonstrating an inaccuracy in the theory of Grüss.

² De Rey-Pailhade: *Bull. général de Thérapeutique*, 142, 17. See also "Rôle du phyllothon dans le mécanisme de l'action des médicaments," *Ibid.*

³ J. A. Vandevelde: "La catalase," *Bull. Soc. Médecine de Gand* (1901); Over de vers preidino von catalase. Pozzi-Escot: "Sur un élément diastatique nouveau de l'urine," *Compt. rend.*, 134, 1332; *Ann. et. Rev. Chim. analyt.* 7, 212; *Ann. Soc. Médecine de Gand*, June 5, 1902.

for this abundance. May it not be due, in part, to their action on hydrogen peroxide? It is known that this is a powerful antiseptic, the presence of which in the cells would be sure to kill them.¹ According to my supposition, the reductases in the cells destroy all traces of this substance as soon as it is formed, and before its presence becomes harmful.

It cannot be denied that hydrogen peroxide is probably formed in the cell during respiration. Recent experiments have proved that labile hydrogen atoms in an organic compound are able to form hydrogen peroxide when in contact with free oxygen.² If it is really produced under these conditions, the reductases must prevent its accumulation by destroying it, and the oxygen set free will be used in the process of respiration.

The protoplasm of the cell is of a very complex organization composed of easily modified proteids. The easy transformations into relatively stable substances during the processes of assimilation and disassimilation are due to the loss of labile atomic groupings by molecular migration, and to a loss of kinetic energy. This energy is transmitted by the protoplasm to thermogenic substances, principally sugars and fatty substances (lecithine), which cannot be directly oxidized at the ordinary temperature. But in contact with the protoplasm these substances can be directly oxidized, for the protoplasm imparts to their constituent atoms so much energy that their reciprocal affinities are augmented, and they become able to combine directly with oxygen, without the necessity of its being first changed into a more active form. The breaking-down of the oxygen molecule, and the union of its atoms with the labile atoms of the sugars and fatty substances, take place at this moment. But, at the same time, certain atoms of hydrogen and thermogenic substances that have become labile

¹ See Beaudet: "De l'eau oxygénée et de ses emplois," *Bull. Chim. de Sucrerie et de Distillerie*, 1898-1899, p. 924. Macé: *Traité de Bactériologie*, 4^e Edit. Des agents chimiques, p. 66. Bert et Regnard: "Influence de l'eau oxygénée sur la fermentation," *Compt. rend.*, 94, 1383 (1882).

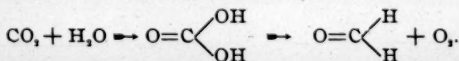
² See Bamberger: *Ber. d. chem. Ges.*, 33, 113; W. Manchot: "Ueber freiwillige Oxydation," *Leipzig*, 1900. Engler: *Ber. d. chem. Ges.*, 33, 1103. Baeyer: *Ibid.*, 33, 1582. O. Loew: "Die chemische Energie der lebenden Zellen," München, 1899. Reinke: *Botan. Zeitung*, 41, 66, 90 (1883).

and charged with energy, may combine with the remaining molecules of oxygen, thus forming hydrogen peroxide as a secondary product. The heat produced by the combustion of the thermogenic substances is transformed to a certain extent into chemical energy by the living protoplasm. This energy facilitates the changes of valence of the atoms, for it increases the amplitude of their vibrations.

By analogous theoretical considerations, it would be possible to show that the respiration of the tissues can be regarded as partly due to the preliminary formation of hydrogen peroxide.

An ingenious theory of Erlenmeyer will serve to explain my idea of the assimilation of carbon in plants. Is the formation of carbohydrates in plants closely connected with the presence of enzymes like the reductases? Such a thing would not be surprising,¹ and if we will recall the facts that have been given, it will be easily seen by what an unbroken series of logical deductions we are led to consider this hypothesis as plausible, *à priori*, and even as well established. The carbohydrates are synthetic products resulting from the general nutrition of the green cells of plants, or just the cells in which the enzymes are produced.

It is generally admitted that the oxygen is set free by the plant by a reaction somewhat like the one below, where we see the carbon dioxide combining with a molecule of water, and forming first formic aldehyde and then oxygen.²

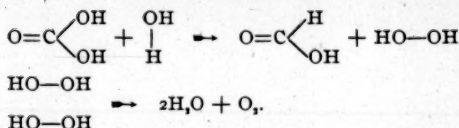


Erlenmeyer tried to explain the separation of oxygen by the intermediate formation of hydrogen peroxide,³ and regarded formic acid as the first product in the assimilation.

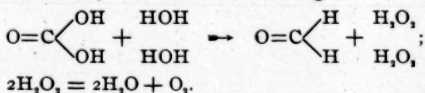
¹ It may be that the enzymes that cause the formation of the sugars, and which Emile Barbet has called (hypothetically) "saccharogenic," are quite different. This seems to be very probable. See E. Barbet: "Sur l'hypothèse d'une diastase saccharogénique," *Bull. chim. de Sucrerie et de Distillerie*, p. 634, 1898-1899; pp. 582, 839, 1897-1898.

² P. Mazé: "L'assimilation du carbone et de l'azote dans le monde vivant," p. 33.

³ Erlenmeyer: *Ber. d. chem. Ges.*, 10, 634 (1877).



However, the formation of formic acid is not at all certain, and does not appear very probable,¹ so that, for the preceding equations, we may substitute the following:²



In conclusion, all that precedes is hypothetical, but the catalytic properties of the reductases, their abundance in all growing organs, the more than probable formation of hydrogen peroxide, and its harmful action, may support my explanations.

All the theories have, in common, the formation of formic aldehyde which polymerizes to form saccharose. The volume of oxygen set free corresponds to the volume of carbon dioxide, the carbon of which entered the formic aldehyde. The theories have not been verified experimentally, although the work of Fischer on the synthesis of the sugars gives them a solid basis of fact. I have shown in what way the catalytic power of the reductases might exert itself. Is this catalyzing force the only one that intervenes, and do the reductases not reduce in the presence of other substances? *A priori*, this seems plausible. It must be admitted that formic aldehyde is not always found in the leaves. Does it polymerize at once, as seems probable from its toxic properties? Or might it not at once form an albuminoid compound, with the properties of an aldehyde, which would be the reductase in question, and would not be toxic? This formation of aldehydes is to be compared with the reduction of the nitrates or of the ammonium salts, whence, by mutual reaction, the nitrogen enters the organism, and quaternary compounds are formed.

¹ Pfeffer; "Physiology of Plants," 1, 356 (1900).

² M-Emm. Pozzi-Escot: "Sur une nouvelle diastase," *Rev. générale de chim.*, 6, 182, 194.

Abundance of the Reductases in Nature.

The reducing enzymes are very abundantly distributed in the animal and vegetable kingdoms. De Rey-Pailhade showed how frequently philothion occurs in animal tissues,¹ by estimating the amounts of hydrogen sulphide set free by them when in contact with an excess of sulphur. Nearly all the organs contain philothion. The white of egg deserves especial mention. When ground with sulphur it at once yields a great deal of hydrogen sulphide. If the white be treated with alcohol it coagulates, and no longer produces hydrogen sulphide when in contact with sulphur.

The yolk, on the contrary, yields no hydrogen sulphide with sulphur alone, and its amount is increased at the first instant by the addition of alcohol.

The reductases exist in the blood, where they attach themselves to the fibrin, to which they impart catalytic properties.

I have also shown that the reductases and Loew's catalase are identical. Loew found his enzyme in a number of plants, some of which had been dried for a long time. A very complete résumé of these experiments was published by the Department of Agriculture in Washington, in 1901. A review of it was published by me.² I verified personally the statements of Loew, and showed that reductases are to be found in all growing tissues.

Fruits contain reductases. The pulp of acid fruits is generally poor in reductases, but the seeds may be very rich in them. It has also been found that in oily seeds the proportion of enzyme is greater than in the others. During germination the proportion of enzyme is greatly increased. In the molds large quantities of reducing enzymes are often met with. These enzymes are relatively very vigorous, and they keep well in solution in glycerol, or in weak alcohol, in the presence of antiseptics. In time they are destroyed by the absorption of oxygen.

Jacquemase, which I discovered in koji (Japanese yeast),³

¹ De Rey-Pailhade: "Recherches experimentales sur le philothion," (1891).

² M.-Emm. Pozzi-Escot: "Sur une nouvelle diastase, la catalase; son rôle dans la nature," Rev. gén. de chim., 8, 182, 194.

³ M.-Emm. Pozzi-Escot: "Sur une nouvelle diastase réductrice, extraite du Koji Japonais et secrétée par l'*Eurotium Oriza*," Compt. rend., 134, 758; Bull. soc. chim., 27, 557.

is secreted by *Eurotium Orizæ*. It is easily distinguished from philothion, because it does not give hydrogen sulphide when in contact with sulphur. I have called it jacquemase in honor of G. Jacquemin, in whose laboratory I discovered it. It has all the properties of the other reductases except the power to form hydrogen sulphide in the presence of free sulphur. It changes nitrobenzene into aniline to a considerable extent. It is very abundant in nature, and especially exists in many tubers.

Béhal, of Paris, suggested calling it "*eurotase*" to recall its origin, in conformity with the principles of classification proposed by Em. Bourquelot. It did not seem necessary to do this, for jacquemase, which was discovered in *Aspergillus Orizæ*, is not confined to that mold, but is also found in a large number of others, and exists in abundance in many of the higher plants. Besides, the name "*eurotine*"¹ has already been suggested for the liquefying enzyme of *Aspergillus Orizæ*. *Eurotine* thus becomes a *eurotase* (Duclaux). In order to avoid this regrettable confusion, it seems to me more practicable to retain the first name.

LABORATOIRES DE RECHERCHES DE
CHIMIE PURE DE G. JACQUEMIN,
MALZÉVILLE, FRANCE.

THE CATALYTIC DECOMPOSITION OF HYDROGEN PEROXIDE.

PART II.

(Continued from the May Number.)

BY J. H. KASTLE AND A. S. LOEVENHART.

AS TO THE MODE OF ACTION OF HYDROGEN PEROXIDE AS AN OXIDIZING AGENT, AND ITS CATALYTIC DECOMPOSITION BY VARIOUS SUBSTANCES.

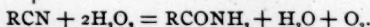
Any satisfactory inquiry into the manner in which hydrogen peroxide acts as an oxidizing agent must take into account the following facts:

1. Hydrogen peroxide is capable of oxidizing certain substances directly and without the intervention of an oxygen

¹ Neuville: "Les ferments industriels d'Extrême Orient," p. 113.

carrier,¹ and without the evolution of oxygen gas. Among the substances which it can thus oxidize may be mentioned the following: Sulphurous acid, ammonium sulphocyanate,² phenolphthalin, and formic aldehyde. We have elsewhere pointed out that, whereas, at low temperatures formic aldehyde is oxidized by hydrogen peroxide with evolution of small amounts of oxygen, at high temperatures this oxidation proceeds very rapidly and without the formation of molecular oxygen.³

2. In certain oxidations by hydrogen peroxide, especially in the presence of oxygen carriers, oxygen gas is evolved simultaneously with the oxidation, and in certain instances, notably in the oxidation of the nitrils, as has been pointed out by Radziszewski,⁴ the same quantity of oxygen leaves the system as molecular oxygen as that which remains in it in combination in the products of the oxidation. For example, according to this observer, the oxidation of a nitril by hydrogen peroxide in alkaline solution takes place in the following manner:



3. A large number of oxidations, which cannot be effected by hydrogen peroxide alone, can be readily brought about by this oxidizing agent in the presence of oxygen carriers. Numerous instances of this sort of change might be given. A good example is furnished in the case of the oxidation of formic acid studied by us, the results of which are given in Part I. of this paper, p. 434.

¹ By an oxygen carrier is here meant a substance which will enable hydrogen peroxide to effect certain oxidations which cannot be accomplished at all, or at least only slowly, by the hydrogen peroxide alone. Such a substance is ferrous sulphate.

² We have observed that ammonium sulphocyanate is very readily oxidized by hydrogen peroxide at ordinary temperatures, with the production of acid ammonium sulphate and hydrocyanic acid.

It has been observed also that the mixtures of ammonium sulphocyanate and hydrogen peroxide give a blue color with guaiacum, thereby indicating that ammonium sulphocyanate can act as an oxygen carrier in the presence of hydrogen peroxide. It has been found also that a solution of ammonium sulphocyanate undergoes a gradual oxidation on exposure to the air, giving rise to a small amount of a reddish substance, which imparts a pink color to the solution. It is well known that solutions of ammonium sulphocyanate gradually acquire a pink color. Such solutions have been found to turn guaiacum blue directly.

³ J. Am. Chem. Soc., 21, 262 (1899).

⁴ Ber. d. chem. Ges., 18, 355 (1885).

Fenton¹ and his co-workers have also investigated a large number of these cases. They have shown that a large number of polyacid alcohols and polybasic organic acids, such as tartaric, glycolic, lactic, glyceric, etc., while not acted on by hydrogen peroxide alone, are readily oxidized by it in the presence of ferrous iron. Emil Fischer has also employed ferric acetate in effecting the oxidation of certain compounds in the sugar group by means of hydrogen peroxide.

Brode² has also pointed out that ferrous sulphate and molybdic and tungstic acids enormously increase the rate of oxidation of hydriodic acid by hydrogen peroxide.

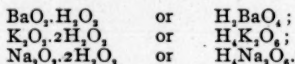
4. Hydrogen peroxide apparently acts as a reducing agent under certain conditions. Under this head may be mentioned its action on silver oxide, as the result of which metallic silver is formed. Instances of this are also furnished by the formation of manganous and plumbous chlorides as the result of the action of hydrogen peroxide on manganese peroxide and lead peroxide in hydrochloric acid solution, and by its action on potassium permanganate in acid solution.

5. It has been pointed out above that those substances which have the power of inducing oxidations by means of hydrogen peroxide are also capable of effecting its catalytic decomposition under certain conditions. In fact, oxidation by hydrogen peroxide in the presence of a catalyzer and its catalytic decomposition often go hand in hand, so that the latter process, as has been pointed out by Spitzer, is often a measure of the former. In our opinion all of these phenomena, widely differing as they appear at first sight, are in reality closely correlated, and referable to a common cause, namely, the tendency on the part of hydrogen peroxide to unite directly with oxidizable substances, thereby forming either a peroxide or some other complex, unstable holoxide derivative, which tends to part with its oxygen more easily than the hydrogen peroxide itself. This derivative, therefore, readily oxidizes some other substance, to which it stands in the relation of an oxygen carrier, or gives

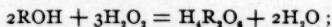
¹ Fenton and Jackson: *J. Chem. Soc. (London)*, **75**, 1 (1899); Fenton and Jones: *Ibid.*, **77**, 69 (1900).

² *Ztschr. phys. Chem.*, **37**, 257 (1901).

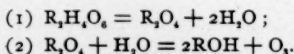
up molecular oxygen, or both. It is important for this assumption, of course, that such higher oxides and unstable addition-products of hydrogen peroxide with other substances should actually exist. In reality, we have abundant proof of their existence. In fact, as may be seen from the following, many such compounds are known. In 1878 Schöne¹ isolated a number of compounds of hydrogen peroxide and the alkalies and alkaline earths, such as the following :



All of these compounds were found by this observer to be very unstable, and are obtainable in a state of purity only at very low temperatures. He therefore explained the catalytic decomposition of hydrogen peroxide by alkalies by the assumption that these hydrogen peroxide addition-products with the alkalies are first produced, thus :



and that these substances then undergo the following transformations :



He was led to assume the intermediate production of such peroxides as R_2O_4 , for the reason that the compounds such as $\text{H}_2\text{K}_2\text{O}_6$ turned yellow during their decomposition.

The work of Schöne on these complex addition-products of hydrogen peroxide has been confirmed by the later researches of Forcrand,² who has obtained evidence not only of the existence of such compounds as H_2BaO_4 and H_2CaO_4 , but also of still more complex derivatives, such as $\text{CaO}_2 \cdot 10\text{H}_2\text{O}_2$.³

¹ Ann. Chem. (Liebig), 192, 257; 193, 241 (1878).

² Compt. rend., 130, 716 (1900).

³ Forcrand represented the compound $\text{CaO}_2 \cdot \text{H}_2\text{O}_2$ by the formula $\text{Ca}(\text{O}-\text{OH})_2$, thereby indicating that it sustains the same relation to hydrogen peroxide, $\text{H}-\text{O}-\text{O}-\text{H}$, that calcium hydroxide, $\text{Ca}(\text{OH})_2$, does to water, $\text{H}-\text{O}-\text{H}$. This is in harmony with the idea first advanced by Traube, that in slow oxidation the molecule of oxygen (O_2) can function as the atom, and that in the true peroxides we have a class of compounds in which the molecule of oxygen (O_2) takes the part of the atom in the oxides. For these compounds Traube proposed the name "holoxides."

Similarly, Berthelot¹ observed that on adding hydrogen peroxide to potassium dichromate in the presence of dilute ammonia, a yellow precipitate was obtained containing hydrogen peroxide in combination with chromic oxide and chromic anhydride, and Moissan,² from his investigation of

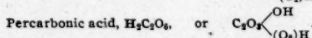
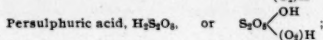
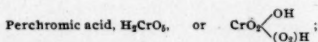
It would seem that the value of this classification and the importance of this idea have not been generally recognized. As a matter of fact, however, it not only affords a very satisfactory means of classifying the peroxides, but it also points to the existence of a large number of derivatives of hydrogen peroxide, which sustain to this substance essentially the same relation that the oxides and hydroxides sustain to water. The following compounds are examples of this kind of combination. For the sake of clearness they are compared with the oxides and hydroxides in the following scheme of classification :

Atomic oxygen, O.		Molecular oxygen, O ₂ .	
Water, H ₂ O.		Hydrogen peroxide, H ₂ (O ₂).	
Oxides.	Hydroxides.	Holoxides.	Hydroholoxides.
M.O	M.OH	M ₂ (O ₂)	M. ₂ ((O ₂)H)
K ₂ O	K.OH	K ₂ (O ₂)	KH((O ₂)H) ₂
Na ₂ O	NaOH	Na ₂ (O ₂)	Ca((O ₂)H) ₂
CaO	Ca(OH) ₂	Ca(O ₂)	Na ₂ H((O ₂)H) ₃
PbO	Pb(OH) ₂	Pb(O ₂)	
Ag ₂ O	AgOH	Ag ₂ (O ₂)	
BaO	Ba(OH) ₂	Ba(O ₂)	

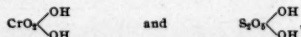
According to this view of the nature of the peroxides, perchromic, persulphuric, and percarbonic acids and their salts are to be regarded as mixed compounds belonging both to the hydroxides and the hydroholoxides and referable to the type



Thus we have :



It will be observed that the formulas proposed for perchromic and persulphuric acids correspond exactly with those in general use for chromic and pyrosulphuric acids,



the only difference being that these peracids contain in the place of one of the hydroxyl groups a hydroholoxide group, (O₂)H, which latter allies them to hydrogen peroxide and affords an explanation of their powerful oxidizing properties. From these considerations one would naturally be led to expect the existence of a corresponding holoxide and hydroholoxide for every oxide and hydroxide now known. When we consider the great instability of these compounds, however, it is scarcely to be wondered at that comparatively so few of these compounds are known. That they are formed in the processes of autoxidation and in reactions with hydrogen peroxide can scarcely be denied, and we have only to admit their formation in order to explain practically all the complex phenomena of autoxidation, oxidation by hydrogen peroxide, and the effect of oxygen accelerators thereon.

¹ Compt. rend., 108, 477 (1889).

² *Ibid.*, 97, 96 (1883).

the unstable blue compound of chromic anhydride and hydrogen peroxide, reached the conclusion that it must be represented by the formula H_2CrO_5 , or $CrO_5 \cdot H_2O$.

Similarly, in order to explain the great acceleration caused by small amounts of molybdic and tungstic acids in the oxidation of hydriodic acid by hydrogen peroxide, Brode¹ has been led to assume the formation of permolybdic and pertungstic acids as the result of the action of the peroxide on the catalyzer, and in the same year Camerer obtained complex peracids of molybdenum and tungsten, to which he assigned these formulas, respectively: $2MoO_5 \cdot H_2O \cdot H_2O$, and $WO_5 \cdot H_2O \cdot H_2O$. It has also been recently shown by Job² that cerous salts can act as oxygen carriers in the presence of hydrogen peroxide, probably through the alternate formation and decomposition of cerium trioxide.

Quite recently Petrenko,³ while studying the action of hydrogen peroxide on sodium arsenate, obtained a compound having the composition $Na_3AsO_5 \cdot 21H_2O$. This he found to be a hydrogen peroxide derivative having the following constitution: $3Na_3AsO_5 \cdot 5H_2O \cdot 16H_2O$. So also has Kazanesky,⁴ obtained the compounds $K_2CO_3 \cdot 2H_2O \cdot \frac{1}{2}H_2O$ and $K_2CO_3 \cdot 3H_2O$. Similarly, Staedel⁵ has recently found that on adding a 90 to 95 per cent solution of hydrogen peroxide to a concentrated aqueous solution of cadmium chloride, a compound was obtained which crystallized in silky needles. It was found to contain 23 per cent of hydrogen peroxide, the theoretical for $CdCl_2 \cdot 2H_2O$, being 27.09 per cent.

Jones and Carroll⁶ have recently investigated the lowering of the freezing-point of certain salts and acids in aqueous hydrogen peroxide as a solvent. In discussing the abnormal lowering of the freezing-point of certain of these salts, such as potassium nitrate, in this solvent, these observers make this significant statement: "The most probable explanation of the above results, all things considered, seem to us to be that

¹ Ztschr. phys. Chem., 37, 257 (1901).

² Compt. rend., 134, 1052 (1902).

³ J. russ. phys. chem. Soc., 34, 391 (1902).

⁴ *Ibid.*, 34, 388 (1902).

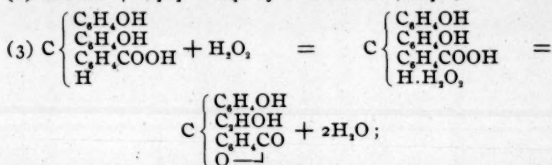
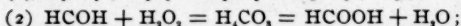
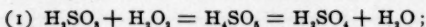
⁵ Ztschr. angew. Chem., 15, 642 (1902).

⁶ This JOURNAL, 28, 284; see also *Ibid.*, 27, 22 (1902).

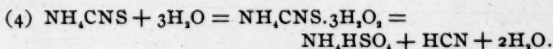
there is union between the molecules of the salts investigated and the molecule of hydrogen peroxide."

There seems to be abundant evidence, therefore, that hydrogen peroxide is not only able to form peroxides, but that it also frequently combines directly with these to form complex, unstable addition-products, and that oxidations by means of this substance are largely, if not entirely, brought about by the formation and subsequent decomposition of these complex compounds. Such being the case, it is believed that we have a simple and intelligible explanation of all oxidations that can be accomplished by this substance :

1. In the oxidation of such substances as sulphurous acid, formic aldehyde, phenolphthalin, and ammonium sulphocyanate, etc., one or more of the molecules of hydrogen peroxide add themselves to these several substances in exactly the proportion required to bring them to their highest labile, holoxide condition of oxidation, the complex derivatives thus produced then breaking down into water and the highest stable oxidation-product of the substance in question. Thus :

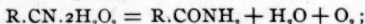


and



2. It is conceivable that certain substances might combine with a larger amount of hydrogen peroxide than that required to effect their oxidation to the highest stable state, in which event they might act as oxidizing agents toward a second substance, and hence as a carrier of oxygen from hydrogen peroxide to the second substance, or their holoxide derivatives

might undergo decomposition with the evolution of oxygen gas. In this category would naturally fall such an oxidation as that of the nitrils, to which reference has been made above. If $R.CN$ were to unite with 2 molecules of hydrogen peroxide we would have $R.CN.2H_2O_2$. This would then decompose in the sense of this equation:



or, as a small amount of alkali is necessary to this oxidation, it is probable that the hydrogen peroxide first combines with this to form a compound like that obtained by Schöne, $H_4K_2O_6$, and that this then combines with the nitril, the product of this combination decomposing at once into the amide, water, molecular oxygen, and potassium dioxide, and that this last combines with more peroxide to form $H_4K_2O_6$, and that these changes thereby take place over and over again until all of the nitril is oxidized. This would explain why Radziszewski found that only a drop or two of caustic potash solution was required to completely oxidize a solution of cyanogen to oxamide.

3. This same explanation would seem to hold for the action of oxygen carriers generally on oxidations by means of hydrogen peroxide. Either the carrier is first oxidized to an unstable peroxide condition by the hydrogen peroxide, or it unites directly with it, or both changes may occur, producing in any case a complex holoxide derivative which oxidizes certain substances more readily than hydrogen peroxide alone. On the other hand, this complex holoxide compound may also break down, yielding water, molecular oxygen, and the original carrier, or some stable oxidation-product of the latter. This is in harmony with Schöne's explanation of the catalytic decomposition of hydrogen peroxide by the alkalis and alkaline earths, and we have seen in the preceding paragraph that alkalis act as oxygen carriers in the oxidation of the nitrils by hydrogen peroxide. Further, Manchot has pointed out that the oxygen-carrying power of iron salts in the presence of hydrogen peroxide can best be accounted for on the assumption that a peroxide of iron is produced.

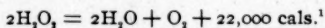
Many reactions exceedingly difficult of explanation thus be-

come readily intelligible in the light of this hypothesis. Take, for example, the action of hydrogen peroxide upon silver oxide and upon metallic silver, concerning which so much has already been written and such widely differing opinions held as to its cause. It is known that silver shows some resemblances to potassium even in the conduct of their oxides. Such being the case, it cannot be far-fetched to assume that when silver or silver oxide is brought into hydrogen peroxide, it is oxidized to Ag_2O_3 , and that then the hydrogen peroxide produces with this a higher holoxide derivative, such as $\text{H}_4\text{Ag}_2\text{O}_4$. We know, however, that silver has but little affinity for oxygen, even in the form of argentous oxide. It is not surprising, therefore, that this more complex compound should decompose easily into water, molecular oxygen, and metallic silver.¹ Or perhaps under slightly different conditions, such as a slight difference in the reaction of the solution, this decomposition might result in the formation of a higher oxide of silver, such as Ag_2O_3 or Ag_4O_5 , as has been contended by Berthelot.² Or if a readily oxidizable substance were present, the silver or silver oxide might act as a carrier of oxygen instead of simply effecting the decomposition of hydrogen peroxide, for the reason that its holoxide derivative would give up part of its oxygen to the oxidizable substance, instead of giving it all up in the form of molecular oxygen. Or both of these processes, namely, the decomposition of the peroxide and the carrying of oxygen, might occur simultaneously. In fact, such is generally the case. The theory here advanced indicates these processes to be correlated phenomena, and the fact that the oxidation of a substance by hydrogen peroxide in the presence of a carrier rarely, if ever, takes place without the decomposition of a portion of the peroxide, is a strong argument in favor of the correctness of this view.

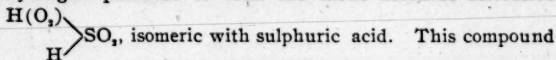
¹ See the work of Baeyer and Villiger on this point. *Ber. d. chem. Ges.*, **34**, 749 (1901).

² In opposition to the views of Thénard and those of Baeyer and Villiger concerning the decomposition of hydrogen peroxide by silver oxide, Berthelot contends that silver peroxide is produced as an intermediate product and that it unites with some of the oxide of silver to produce the compound Ag_4O_5 . *Ann. chim. phys.*, [7], **11**, 223 (1897).

For a thorough understanding of such reactions, it is well to remember that the tendency to form molecular oxygen is no inconsiderable element in the affinities which are at work to bring about these changes. That such is the case is seen by the fact that the change of ozone into oxygen is an exothermic process, and that in the decomposition of hydrogen peroxide into water and molecular oxygen 22,000 cal. of heat are evolved :

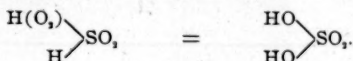


On the other hand, before we can have atomic oxygen available for the purposes of oxidation, it is necessary that energy enter the system either directly or as the result of chemical change. In view of this, and of what has already been given above, there is every reason for supposing that the oxygen evolved in these processes does not result directly from the hydrogen peroxide, but rather indirectly from the decomposition of these complex addition-products of hydrogen peroxide with the oxidizable substance, and that the formation of molecular oxygen as the result of the decomposition of these complex oxygen compounds is essentially similar to the formation of molecular nitrogen from the decomposition of a diazo compound. The ideas here advanced enable us to understand how it is that hydrogen peroxide sometimes acts as an oxidizing agent, without any evolution of oxygen, while under other conditions it oxidizes most rapidly when it is giving off oxygen gas most freely. In the first case, the hydrogen peroxide adds itself to the oxidizable substance to form a labile holoxide derivative, which by rearrangement, and in some instances by splitting off water, furnishes the highest stable oxidation-product of the substance in question. Hence, in such oxidations, no molecular oxygen is evolved. A good example of this mode of action is furnished by the oxidation of sulphur dioxide by hydrogen peroxide. According to our view of the matter, sulphur dioxide combines directly with hydrogen peroxide to form the labile holoxide derivative

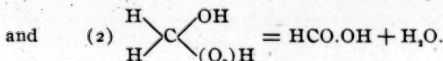
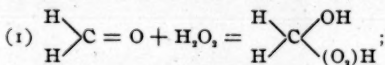


¹ Mendeleeff's "Principles of Chemistry," (1897), Vol. I., p. 208, foot-note 18.

then undergoes molecular rearrangement, and forms sulphuric acid with the evolution of heat. Thus:



Similarly in the oxidation of formic aldehyde by hydrogen peroxide the following changes would probably occur:



It has also been observed that in those oxidations by the peroxide which are more or less dependent on the presence of finely divided platinum or a similar carrier, the oxidation and the catalysis are both affected alike by temperature. At low temperatures oxygen is given off slowly and the oxidation is correspondingly slow, whereas, at higher temperatures, both the catalytic decomposition and the oxidation are greatly accelerated. This correlation of properties on the part of the carrier and the similar effect of the temperature on the two processes, can be readily understood when it is borne in mind that the formation and decomposition of the unstable oxygen compound produced by the action of the peroxide on the carrier is a function of the temperature, and that at low temperatures it would probably be formed less readily and be more stable than at higher temperatures. Hence, at very low temperatures, hydrogen peroxide would probably not be decomposed by platinum black, nor would the latter induce the oxidation of any substance by the peroxide. On the other hand, at higher temperatures, the unstable peroxide addition-product of the carrier and hydrogen peroxide would not only be produced more rapidly, but would also decompose more easily, and hence would oxidize the reducing substance more rapidly. In this manner the conduct of a carrier like platinum black can be satisfactorily explained.

In an inquiry into the cause of oxygen transmission by

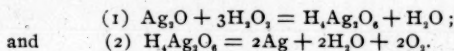
platinum, E. von Meyer¹ decided against de la Rive's hypothesis of alternate oxidation and reduction of the carrier, mainly for the reason that he found PtO , $\text{PtO}(\text{OH})_2$, and PtO_2 to conduct themselves differently from spongy platinum and air towards a mixture of hydrogen, carbon dioxide, and oxygen. He, therefore, rather inclined to the view of Hüfner, that the platinum acts by loosening the affinities of the atoms within the oxygen molecule, thus rendering it more active. According to our conception, von Meyer's results are easily explained. It is more than likely that the oxides of platinum, which he compared with a mixture of air and spongy platinum in their oxidizing power towards a mixture of hydrogen and oxygen, are by no means the same as the highly unstable holoxide compound of the metal and oxygen, which is alternately formed and reduced during the oxidation. Indeed the whole modern theory of oxidation rests upon the assumption, well verified in many cases, that it is not the final products of oxidation that are first formed during the process but very unstable holoxides. These intermediate products may carry oxygen to other less oxidizable substances, or react with water to form hydrogen peroxide, or in some cases give off ozone. The formation of ozone in the slow combustion of phosphorus, for example, is inexplicable by any other assumption, and yet the holoxide of phosphorus, which is the precursor of phosphorus pentoxide and ozone, has never been isolated.² We do not look to phosphorous or phosphoric oxide, however, to explain the oxygen-carrying power of phosphorus, and why should we do this in the case of platinum? Then again, Hüfner's explanation is no explanation at all. The only means of loosening the affinities of an element is by kinetic energy, or by the combination of the substance with another to form an unstable derivative which can accomplish changes impossible to the original substance.

4. In our opinion it is a mistake to look upon the so-called reductions brought about by hydrogen peroxide as reductions in the ordinary sense. In reality they are probably oxidations, at least in their initial phases. In these processes it is

¹ J. prakt. Chem., [2], 14, 124 (1876).

² Ostwald: Ztschr. phys. Chem., 34, 248 (1900).

certainly more easily conceivable that the hydrogen peroxide first oxidizes the substance whose reduction it ultimately effects, or perhaps, adds itself to it, and that the complex compound thus produced then breaks up into the final products of the change. Thus, as indicated above, silver oxide is probably oxidized and combines with hydrogen peroxide after the manner of the alkalis, thus :



In this same way other so-called reductions by hydrogen peroxide can readily be explained by the supposition that the change is primarily one of oxidation. For example, when lead peroxide and manganese peroxide decompose a neutral or alkaline solution of hydrogen peroxide, the oxygen evolved has been found to come entirely from the hydrogen peroxide, and the metallic peroxides are found unchanged at the end of the process. On the other hand, when they react in an acid solution of hydrogen peroxide, they are reduced and are found at the end of the operation as plumbous and manganous salts.¹ We have seen that the catalytic decomposition of the neutral peroxide can be most simply explained by the supposition that unstable holoxides and hydroholoxides are produced, which by their decomposition give rise to the water, molecular oxygen, and the catalyzer in its original form. Hence, it would seem probable that the reduction of these substances, which is effected in acid solutions of the peroxide, is likewise preceded by the oxidation of the manganese or lead peroxides to more complex holoxide derivatives, and that it is upon the latter that the acid acts, with the production of water, molecular oxygen, and an -ous salt of the metal. As a matter of fact, numerous instances are known in which higher and more unstable oxides are more readily acted upon by acids than the corresponding lower and more stable oxides.

Then again, take the action of hydrogen peroxide on an acid solution of potassium permanganate, whereby the permanganate is reduced with the formation of a manganous salt,

¹ Martinon : Bull. soc. chim., 43, 355 (1885).

water, and molecular oxygen. This reaction has been variously interpreted by different observers. According to Berthelot, and later according to Bach, the permanganic acid oxidizes the hydrogen peroxide to hydrogen trioxide, and this undergoes decomposition into water and molecular oxygen. Here one-half of the oxygen given off is furnished by each of these substances. According to Schönbein, atomic oxygen is given off simultaneously in this process from the hydrogen peroxide and the permanganic acid, forming molecular oxygen, whereas, according to Traube and Weltzien, the permanganic acid oxidizes the hydrogen of the hydrogen peroxide to form water, so that the oxygen evolved in this process really comes entirely from the hydrogen peroxide.

Baeyer and Villiger¹ object to Schönbein's explanation that both of the substances concerned act as oxidizing agents. They even go so far as to say that hydrogen peroxide is scarcely to be considered an oxidizing agent since, in pure state, it decomposes dilute hydriodic acid only very gradually. This is certainly a remarkable view to take of the conduct of hydrogen peroxide in the light of all the facts, and one that is certainly not shared by chemists generally. They also object to the views of Berthelot and Bach, that higher oxides of hydrogen are produced by the oxidation of hydrogen peroxide by permanganic acid, on the ground that it does not rest on a sufficiently broad foundation of fact.² They are not prepared, however, to urge the stability of Caro's acid towards permanganic acid as an argument against Berthelot's view.

On the other hand, they incline to the views of Traube and Weltzien as best according with the facts, namely, that in the reaction, the permanganic acid oxidizes the hydrogen of the hydrogen peroxide, and that, therefore, the oxygen evolved comes entirely from the hydrogen peroxide itself, and they go so far as to say that the writers of text-books on elementary chemistry would be justified in adopting this explanation of this change. They have likened the oxidation of hydrogen

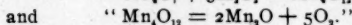
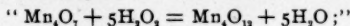
¹ Ber. d. chem. Ges., 33, 2488 (1900).

² Quite recently, however, Baeyer and Villiger (Ber. d. chem. Ges., 35, 3038 (1902)) state that the oxides K_2O_4 , etc., are derived from the acid H_2O_4 , which they call ozonic acid, and of which they consider ozone the anhydride, thus: $O_3 + H_2O = H_2O_4$. This seems to be nothing more than Bach's hydrogen tetroxide. See Bach: Ber. d. chem. Ges., 35, 3424 (1902); Cf. Clover: This JOURNAL, 29, 463 (1903).

peroxide under these conditions to that of hydrogen sulphide, and cite other analogies of hydrogen peroxide to hydrogen sulphide in support of this view.

In our opinion, this analogy rests upon a very uncertain and slender foundation of fact, and even admitting its correctness, it must needs impress us as a little peculiar that, in order to oxidize the hydrogen of hydrogen peroxide to water, the oxygen necessary to effect this oxidation must be supplied from an outside source, when the compound itself contains more oxygen than any other compound, unless it is Bach's hydrogen tetroxide, and when, as is well known, half of this oxygen is readily available for the purposes of oxidation.¹

The view that hydrogen peroxide could oxidize permanganic acid, seems never to have received much attention at the hands of chemists, and yet, as a matter of fact, we have observed that its conduct as an oxidizing agent can be best explained by the supposition that it converts substances upon which it acts into their holoxide forms. According to Brodie,² whose views of oxidations and reductions by hydrogen peroxide were remarkably clear and consistent for his time, the reduction of permanganic acid is probably preceded by an oxidation thus :



It would seem, therefore, that the simplest view to take of the matter is that the hydrogen peroxide converts permanganic acid into a normal holopermanganic acid, having the composition :



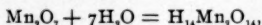
This compound, $\text{H}_{14}\text{Mn}_2\text{O}_{19}$, may be regarded as being derived from the anhydride of permanganic acid, Mn_2O_7 , by the combined action of water and hydrogen peroxide.³ Normal per-

¹ Bach, also, is of the opinion that Traube's view, supported by Baeyer and Villiger, that the peroxide of hydrogen contains easily oxidizable hydrogen, is contrary to reason. *Chem. News*, 85, 220; *Moniteur Scientifique* (4) 17, 26 (1903).

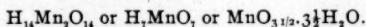
² *J. Chem. Soc. (London)*, 17, 281 (1864).

³ It has been shown previously by one of us, Kastle, that the composition of a great many hydrates of the metallic nitrates can be satisfactorily explained by the assumption that these complex compounds are derived from normal nitric acid, H_2NO_5 , and similar explanations doubtless hold for many other complex compounds now but little understood. *This JOURNAL*, 20, 814.

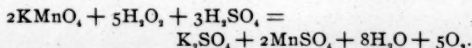
manganic acid is derived from the oxide Mn_2O_3 by the addition of 7 molecules of water, thus :



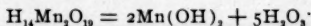
and hence must have the formula



Now assuming that 1 molecule of water enters into the composition of normal holopermanganic acid, and remembering that, in the formation of these addition-products of hydrogen peroxide, the hydrogen peroxide is equivalent to water, it will be observed that 2.5 molecules of hydrogen peroxide will be required to produce normal holopermanganic acid, or for the double formula of permanganic acid 5 molecules of hydrogen peroxide will be required. Now, this is the quantity of peroxide actually required to effect the decomposition of acid permanganate, thus :



In the light of Berthelot and Bach's observations, however, it is probable that the decomposition of normal holopermanganic acid under the influence of acids takes place more gradually than the above equation would indicate. According to our view of the matter, it would probably take place in this way :



This would satisfactorily explain the formation of the higher oxides of hydrogen, and would account satisfactorily for the fact observed by Berthelot, that a mixture of potassium permanganate, sulphuric acid, and hydrogen peroxide at -12° C. becomes colorless, without the evolution of oxygen.

Baeyer and Villiger observed that oxygen is readily evolved from this cold solution by shaking. They, therefore, looked upon the phenomenon as one of supersaturation. As we know, however, oxygen is only slightly soluble in water, hence it is rather difficult to understand how it could form supersaturated solutions containing such large amounts of oxygen. On the other hand, we know that agitation assists

the decomposition of any very unstable compound; so that these facts are much more easily explained by the supposition that a holopermanganic acid is formed and undergoes decomposition than by any other hypothesis. Another point brought out by Baeyer and Villiger is that Caro's acid, persulphuric acid, is much more stable towards permanganic acid than hydrogen peroxide. They account for this by the supposition that easily oxidizable hydrogen becomes more difficult to oxidize by combination with an acid radical, as may be observed in the conduct of phosphine, hypophosphorous, and phosphorous acids towards oxidizing agents.

On the supposition that normal holopermanganic acid is produced by the action of hydrogen peroxide on permanganic acid, we have a more simple explanation of the greater stability of persulphuric acid towards permanganic acid than any that has hitherto been advanced. According to our hypothesis, persulphuric acid and holopermanganic acid are analogous compounds, both being holoacids. Therefore, there would be but little or no tendency on the part of the former to produce the latter from permanganic acid. On the other hand, we have observed that just as water is primarily an agent which effects hydrolysis, so hydrogen peroxide possesses a marked tendency to oxidize a great variety of compounds to the holoxide condition.

It is highly probable, therefore, that all reductions effected by hydrogen peroxide are always preceded by an oxidation of the substances reduced. In other words, that the reduction is of the nature of a secondary process and often tends to disguise the true significance of the reaction.¹

¹ Since this communication went to press similar ideas regarding the apparent reduction of certain metallic compounds by hydrogen peroxide have been advanced by Bach. As the result of his study of the action of chromic acid on hydrogen peroxide, he arrived at the conclusion that superoxygenated derivatives of chromium are produced. The formation of such a compound would, according to him, enable us to explain the decomposition of the peroxide by the metallic oxides more or less rich in oxygen, in a very simple manner. In the case of certain elements, such as uranium, vanadium, titanium, molybdenum, etc., etc., these intermediate compounds are relatively stable and can be isolated; in the case of other elements, such as chromium, the intermediate compound is only slightly stable, but its characteristic coloration places its formation beyond all possible doubt. In the case of other elements again, such as silver, platinum, lead, and manganese, the formation of the intermediate compound is not made manifest by any outward sign. Towards the close of his article he says:

Finally, it is believed that the formation of these unstable holoxide derivatives by the action of hydrogen peroxide on various catalyzers, and their subsequent decomposition into molecular oxygen, water, and the catalyzer in its original form, affords a satisfactory explanation of the catalytic decomposition of hydrogen peroxide by these various substances. According to our view of the matter, these unstable holoxide derivatives may react in two ways. They may either effect the oxidation of certain reducing agents, which cannot be oxidized by hydrogen peroxide alone, or, in the event that such reducing agents are not at hand, these holoxide derivatives may undergo decomposition themselves, yielding molecular oxygen, water, and the catalyzer in its original form, or a stable oxidation-product of the latter. The fact that both of these processes take place, as a rule, simultaneously, and that, generally speaking, a good oxygen carrier is also a good catalyzer, and vice versa, is of itself good evidence that these two processes are causally related, and furnishes one of the strongest arguments in favor of the correctness of this view. Additional evidence of its correctness is afforded by the following observations:

1. It has been observed by Kastle and Clark¹ that when hydrogen peroxide is brought in contact with finely divided copper, a certain interval elapses before the decomposition begins. During this latent period the copper undergoes a marked change of color, passing from a dull-red to a dark-brown or black. As soon as this change of color occurs, the decomposition of the hydrogen peroxide begins actively and continues as long as any of the compound is present.

Now, according to our view of the matter, finely divided copper can effect the decomposition of hydrogen peroxide only

"In the presence of acids the intermediate compound undergoes a more decided decomposition with the evolution of oxygen and the formation of a salt. If the oxide employed contains 'disposable' oxygen, that is to say, oxygen in excess of that required to form a salt with the maximum amount (MnO_2 , PbO_2 , MnO_4H , CrO_3) this oxygen is given off at the same time as that from the peroxide of hydrogen, which causes the illusion of the simultaneous reduction of the metallic oxidizer and the peroxide of hydrogen. As a matter of fact, there is nothing but the more or less spontaneous decomposition of the superoxygenated product, formed by the fixation of the peroxide of hydrogen on the metallic oxide brought into the reaction." *Moniteur Scientifique*, [4], 17, 26. See also *Chemical News*, 87, 32.

¹ This *JOURNAL*, 26, 526 (1901).

by being first converted into some unstable holoxyde derivative. The formation of such a compound by the action of the peroxide on the metal is seen in the change of color which the latter undergoes prior to the decomposition, and the latent period here observed represents the time interval necessary for the formation of this unstable compound.¹ There seems to be no necessity for supposing that these phenomena are peculiar to copper. On the other hand, there is every reason to believe that in the decomposition of hydrogen peroxide by other metals and by various other catalyzers, we have the same succession of events, the conversion of the catalyzer into the holoxyde form, followed by its decomposition into molecular oxygen, water, and the catalyzer, the only difference being that this succession of events takes place more rapidly with most catalyzers than with copper, and without a change of color in most cases.

THE RÔLE OF CATALASE IN THE LIVING CELL.

It was long ago pointed out by Schönbein that one of the most characteristic and universal properties of living protoplasm is its power to decompose hydrogen peroxide. Indeed, he looked upon this decomposition as the prototype of all fermentative activity, and gradually it came to be accepted as a fact that all ferments and enzymes possess this property in common, and correlated with it, the power also to give the guaiacum reaction in the presence of hydrogen peroxide. Strange to say, these ideas still find a place in the recent literature of the subject.

On the other hand, in 1888, Bergengrün² found that fibrin ferment does not decompose hydrogen peroxide, and in 1892

¹ In order to explain the period of induction observed in the combination of moist hydrogen and chlorine, a number of observers have found it necessary to assume the formation of an intermediate compound. Recently Mellor, in a remarkably accurate and painstaking investigation, has shown that this intermediate compound is not Cl_2O or HClO , but a substance the composition of which he represents by the formula $x\text{Cl}_2, y\text{H}_2\text{O}, z\text{H}_2$, where x , y , and z are positive integers. If we assume the existence of such an intermediate compound, then, according to him, the period of induction is a necessary consequence of the law of mass action. *J. Chem. Soc. (London)*, 81, 1292 (1902).

² Ueber die Wechselwirkung zwischen Wasserstoffsperoxyd und verschiedenen Protoplasma Formen, Dorpat, 1888. Cited by Loew: Report No. 68 U. S. Department of Agriculture (1901).

Jacobson¹ observed that by carefully heating emulsin it might be made to lose its power to decompose hydrogen peroxide, without losing its power to hydrolyze amygdalin. It might also be noted in passing that very powerful preparations of certain enzymes have been on the market for a long time, which have but very feeble action on hydrogen peroxide.

In 1897 Spitzer² pointed out that the power to decompose hydrogen peroxide is due to one enzyme only, *viz.*, to that which gives the guaiacum reaction. It has been observed by Loew,³ however, that, while aqueous extracts of cured tobacco have the power of actively decomposing hydrogen peroxide, they did not have the power to hydrolyze albumin, starch, cane-sugar, or amygdalin, and also that such extracts were unable to give the guaiacum reaction, even in the presence of hydrogen peroxide. He therefore reached the conclusion that the power of various animal and plant extracts to decompose hydrogen peroxide is due to a distinct enzyme of general occurrence, to which he gave the name of "catalase." Naturally there has been considerable speculation as to the physiological rôle of catalase in the cell. Schönbein connected it with the process of respiration, *i. e.*, with oxidations occurring in the organism, and in harmony with this idea, Spitzer later reached the conclusion that the power of a given tissue to decompose hydrogen peroxide is a true index of its oxidizing power. On the other hand, it was afterwards shown by Lépine⁴ that for the various tissues there does not always exist this parallelism between the quantities of oxygen evolved from hydrogen peroxide as the result of their action and the intensity of their other reactions, such as the oxidation of guaiacum, guaiacol, etc. Therefore, in attempting to explain the rôle of catalase in the living cell, Loew has found it necessary to depart somewhat from formerly accepted ideas as to its physiological significance. According to this observer, catalase may act beneficially in the cell in three ways:

1. On the supposition that hydrogen peroxide is a constant

¹ Ztschr. physiol. Chem., 16, 340 (1892).

² Pflüg. Arch., 67, 615 (1897).

³ U. S. Dept. of Agr., Report No. 68 (1901).

⁴ Compt. rend. Soc. Biol., 1899, p. 401.

by-product of all respiratory processes, he assumes that the chief function of catalase is to destroy this substance as fast as found, thereby protecting the cell against toxic action.

2. In view of the fact that catalase is found in anaerobic micro-organisms, in which hydrogen peroxide is not produced, he assumes that this enzyme has the property of loosening the affinities in certain other compounds besides hydrogen peroxide, in order that the protoplasm may be able to effect their decomposition more easily. "In other words," he says, "catalase might represent an aid for fermentative as well as for respiratory phenomena."¹

3. He suggests that catalase may be able to effect certain reductions, since platinum-black can bring about both oxidations and reductions.

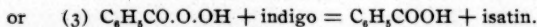
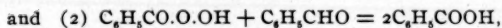
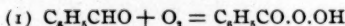
It seems to us that the first suggestion of Loew's, that the chief physiological rôle of catalase is to prevent the accumulation of hydrogen peroxide in the cell, is highly improbable. In the first place, Erlenmeyer's hypothesis that hydrogen peroxide is produced as a by-product in the assimilation of carbon dioxide by green plants is as yet entirely without experimental proof, and it certainly cannot be said to have been proved conclusively that hydrogen peroxide is present either in vegetable or animal tissues, although this in itself would really be no argument against Loew's hypothesis.

As a matter of fact, if hydrogen peroxide is produced at all in respiratory processes, it can only result from the interaction of organic peroxides (oxidases) with water and basic substances. We have endeavored to prove in a former communication² that the so-called oxidases are probably nothing more than unstable organic peroxides, which are produced in the cell as the first oxidation-products of certain autoxidizable substances, and this view is shared by Bach and others. Now, as is well known, these unstable peroxides can either oxidize a second molecule of the substance from which they are derived, or any other strongly reducing substance that may happen to be present. That such is the case is evident from the work of Baeyer and Villiger on the oxidation of benzalde-

¹ *Loc. cit.*, p. 44.

² This JOURNAL, 26, 539 (1901).

hyde.¹ According to these observers, this compound may participate in the following changes :



The organic peroxides or oxidases would, therefore, only react with water, or a basic substance, to form hydrogen peroxide, in case no further quantities of reducing substances were present, and this under normal conditions of life and growth is exceedingly improbable.

Even supposing that small amounts of hydrogen peroxide should be produced by the action of water on the organic peroxides, it is known that associated with catalase there are enzymes or substances present in the animal and plant cell which can act as oxygen carriers and enable the hydrogen peroxide thus produced to effect certain oxidations. This is evident from the so-called peroxidase reactions. An extract of liver, for example, does not oxidize either guaiacum or phenolphthalin directly, but in the presence of hydrogen peroxide both of these substances are oxidized, and at the same time the hydrogen peroxide is rapidly decomposed. Hence it is more than probable that even if hydrogen peroxide were produced in the cell, it would be at once turned to account for the process of oxidation.

Finally, even admitting that hydrogen peroxide should be produced in the cell as the result of any of these reactions, and that it could not be turned to account in the manner indicated, it is still difficult to conceive that the rôle of catalase should be to destroy this substance as a safeguard against its toxic action, when, keeping in mind the peroxide nature of the oxidases, we remember that, as has been recently shown by Freer and Novy,² the organic peroxides, particularly of the asymmetrical type, are much more powerful antiseptics than hydrogen peroxide itself. Certainly all of these facts should be taken into account, and if they are, it is hardly conceivable

¹ Ber. d. chem. Ges., **33**, 1569 (1900).

² This JOURNAL, **27**, 161 (1902).

ble that the chief rôle of catalase in the cell is to destroy hydrogen peroxide.

On the other hand, the fact that the two modifications of catalase, in the absence of other oxidases, can oxidize hydroquinone, as has been proved by Loew, would seem to indicate that catalase is really an oxidase. This is in harmony with the fact that most, if not all, substances which can break down hydrogen peroxide, can also effect the oxidation of various substances in contact with molecular oxygen. Platinum-black, other finely divided metals, and bone-black are substances of this kind.

In this connection it should also be borne in mind, as has been pointed out above, that those substances which are able to effect the decomposition of hydrogen peroxide, can also, under suitable conditions, act as oxygen carriers in the oxidation of various substances by the peroxide. Apparently catalase is an exceptional catalyzer, in the sense that while it readily decomposes the peroxide, it, according to Loew, is incapable of transmitting oxygen from the peroxide to guaiacum. In other words, catalase, when free from other enzymes, does not give the peroxidase reactions.

In spite of its exceptional conduct in this respect, and in the light of Loew's result showing that catalase can oxidize hydroquinone, we are inclined to Schönbein's view that the substances in the living cell which are capable of effecting the decomposition of hydrogen peroxide, are concerned in some way with oxidation phenomena, that is, if these substances have any physiological rôle at all.

From the facts as they are known to us at present, it would seem most logical to look upon catalase as a substance or mixture of substances capable of combining with oxygen to form an oxidase, and with hydrogen peroxide to form an unstable holoxide derivative. This would explain its oxidizing action on hydroquinone and at the same time would offer a satisfactory explanation of its power to decompose hydrogen peroxide, and would put it in the same class with other hydrogen peroxide catalyzers. The fact that it does not oxidize guaiacum in the presence of hydrogen peroxide is not surprising

when we recall the specific character of the oxidations brought about by oxidases. Tyrosinase, for example, will oxidize tyrosine, but not salicylic aldehyde, and aldehydase will oxidize salicylic aldehyde, but not tyrosine. In other words, a number of oxidizing ferments are known which seem to be specific in their oxygen-carrying powers. It should also be borne in mind in this connection that both Aso¹ and Bach,² independently, have reached the conclusion that at least two peroxidases occur in plants.

From our standpoint, therefore, the power possessed by catalase to decompose hydrogen peroxide is to be looked upon, not as its physiological function, but rather as a property possessed in common by a great many substances capable of combining with oxygen, a property, by the way, which may never be called into play physiologically.

The idea that probably catalase is a reducing substance is shared by others, and by Loew himself. Recently Pozzi-Escot³ has shown that, in most respects at least, it is identical with the "philothion" of de Rey-Pailhade. As is well known, this last-named substance belongs to that class of soluble ferments known as the reductases.

Now, according to de Rey-Pailhade⁴, philothion should be able to combine with oxygen as well as with sulphur, and this, according to him, is its principal function. Pozzi-Escot has pointed out that the action of philothion or catalase on hydrogen peroxide is not strictly catalytic, but that it at length ceases, owing to the oxidation of the substance. This is just what we should expect if our view of the nature and function of catalase is correct.

Summary of Results.

The results and conclusions reached in this investigation may be briefly summarized as follows:

1. It has been found that in many instances at least the inhibition of the catalytic decomposition of hydrogen peroxide by various metals is due to the formation of thin, insoluble,

¹ Bulletin College of Agriculture, Tokyo, 5, 2, 218 (1902).

² Ber. d. chem. Ges., 36, 605 (1903).

³ Bull. soc. chim., [3], 27, 280 (1902); This JOURNAL, 29, 552 (1903).

⁴ Compt. rend., 106, 1683; 107, 43 (1888); 108, 356 (1889).

protective films over the surface of the metal, the formation of which is brought about by the action of the inhibitor on the metal. In our opinion the inhibitory effect of these substances, many of which happen to be poisons, in nowise indicates any real analogy between the finely divided metals and the soluble ferments. In our opinion, therefore, the use of the term "platinum poison" in this connection, is altogether misleading and fallacious.

2. It has also been pointed out that, during the decomposition of hydrogen peroxide by various catalyzers, we have no evidence of the formation of atomic oxygen. It has been found, however, that various catalyzers act as oxygen carriers in the presence of hydrogen peroxide, and that, with the exception of catalase, all substances which break down hydrogen peroxide also act as oxygen carriers, and *vice versa*, and that for any given catalyzer the oxidation is proportional to the rate of catalytic decomposition.

From these facts, supported also by much collateral evidence, we have been led to conclude that these two properties, *i. e.*, that of inducing oxidation by hydrogen peroxide and that of effecting its catalytic decomposition, are correlated, and that both depend on the fact that the catalytic agent is capable of reacting or combining with the hydrogen peroxide to form complex unstable holoxide derivatives as intermediate products. In the presence of suitable reducing substances these unstable holoxide derivatives may act as oxidizing agents, or, in the absence of the same, they may undergo spontaneous decomposition into molecular oxygen, water, and the catalyzer in its original form, or an oxidation-product thereof, or, as happens in most cases, if suitable reducing substances are present, both oxidation by the holoxide derivative and its spontaneous decomposition may occur simultaneously.

In keeping with these views, the idea has been advanced that the so-called reductions by means of hydrogen peroxide are always preceded by oxidations, and that in the strict sense of the term hydrogen peroxide is not a reducing agent at all.

3. In the light of these conclusions it would seem that the

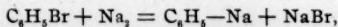
physiological function of Loew's catalase is not to effect the decomposition of hydrogen peroxide as a means of protecting the cell against the toxic action of this substance, but rather that catalase is a substance or mixture of substances having a tendency to combine with hydrogen peroxide to form a very unstable holoxide derivative, which immediately decomposes with the evolution of molecular oxygen in the manner above described for other holoxides. We would not confuse this property, however, with its physiological function. Rather are we inclined to look upon catalase as a reducing substance, which may under some conditions combine with atmospheric oxygen to form an oxidase,¹ or as Pozzi-Escot² has pointed out, a substance probably identical with de Rey-Pailhade's philothion, which, under certain conditions, can effect reductions.

STATE COLLEGE OF KENTUCKY,
LEXINGTON, KY., Nov., 1902.

Contribution from the Kent Chemical Laboratory of the University of Chicago.
ON SODIUM PHENYL AND THE ACTION OF
SODIUM ON KETONES.

BY S. F. ACREE.

The following work was undertaken at the suggestion and under the direction of Dr. J. U. Nef. Its object was to prepare sodium phenyl and to ascertain whether it is formed as an intermediate product in various reactions in which bromobenzene and sodium are used. Nef came to the conclusion, from his experiments with sodium phenylacetylene, that sodium phenyl must first be formed in every case,³



and that this substance subsequently reacts with other reagents that may be present, as alkyl haloids (Fittig), ketones (Frey),⁴ carbon dioxide (Kekulé), chlorcarbonic ether (Wurtz).

We have since succeeded in preparing sodium phenyl from mercury phenyl and sodium. It reacts with alkyl, aryl, and

¹ U. S. Dept. of Agr., Rep. No. 68, 39-40.

² Bull. soc. chim., [3], 27, 280 (1902); This JOURNAL, 29, 552 (1903).

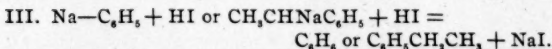
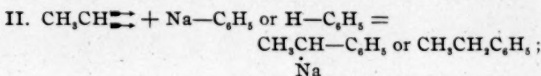
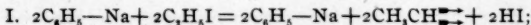
³ Nef: Ann. Chem. (Liebig), 308, 281, 291.

⁴ Frey: Ber. d. chem. Ges., 28, 2514.

acyl halides, with ketones and other reagents, giving practically the same products which are formed when these reagents are treated in ethereal solution with brombenzene and sodium; hence it may be concluded that in those reactions where brombenzene and sodium are used (Fittig's, Frey's, Kekulé's, Wurtz's reactions), sodium phenyl is the intermediate product.

Nef has shown¹ that the reactions of the primary and secondary alkyl haloids with other substances such as alcoholates, amines, etc., depend upon the dissociation of the alkyl haloid into alkylidene and halogen hydride. The halogen hydride is neutralized by the alcoholate, amine, etc., while the alkylidene absorbs the alcohol, amine, etc., that is present, by addition (alkylation). In some cases the alkylidene may also, in part, rearrange to an olefine or polymerize to a dialkylidene.

Sodium phenyl should, from this point of view, interact with primary and secondary alkyl haloids, *e. g.*, ethyl iodide, as follows:



Part of the ethylidene may also go over into ethylene by intramolecular alkylation, or in the case of benzyl chloride the phenylmethylene may polymerize in part to stilbene.²

These expectations were in every way realized. It is surprising with what intense violence the interactions of sodium phenyl take place even with an inactive substance like brombenzene.

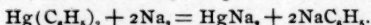
Preparation of Sodium Phenyl.

When mercury diphenyl is dissolved in dry benzene or ligroin and treated in a flask with a return-condenser, with

¹ Ann. Chem. (Liebig). 309, 126, 318, 2.

² Nef: *Loc. cit.*

considerably more than the molecular quantity of fine sodium wire, the sodium reacts vigorously with the mercury diphenyl. Sodium amalgam is formed and falls to the bottom of the flask, while a light-brown powder, sodium phenyl, remains suspended in the solution. The larger the amount of sodium used, the more nearly complete is the reaction,



That this powder is sodium phenyl and not sodium mercury phenyl, corresponding to sodium zinc ethyl,¹ was proved by analysis. One sample was made up especially for analysis, as follows: Five grams of mercury diphenyl, dissolved in 100 cc. of benzene, were boiled with five grams of sodium wire until the reaction was complete. Upon adding 2 grams of sodium wire and boiling again there was hardly any action. This showed that very little mercury diphenyl had failed to react with the first portion of sodium. As sodium phenyl is very readily decomposed by the moisture of the air, the analysis was accomplished as follows: A 50 cc. dropping-bottle was fitted with a stop-cock and a stream of dry hydrogen was passed through the bottle; it was then weighed. Part of the sodium phenyl, together with some benzene, was withdrawn from the flask by means of a pipette and transferred to the dropping-bottle. The benzene was then distilled from the dropping-bottle in a stream of hydrogen, leaving behind the sodium phenyl as a dry powder. The dropping-bottle was again weighed, the difference between the two weights giving the amount of the sodium phenyl. When water was added to the dropping-bottle the sodium phenyl was decomposed, forming sodium hydroxide and benzene. The sodium hydroxide was titrated with N/1 sulphuric acid and phenolphthalein. There was a small amount of mercury diphenyl, insoluble in water, left in the dropping-bottle. This showed that the sodium phenyl was not quite pure, and accounts for the low results obtained in the analyses.

Analysis of first preparation:

I. 0.5793 gram substance required 5.70 cc. N/1 sulphuric acid.

¹ Wanklyn: *Ann. Chem. (Liebig)*, 108, 67; *Z.*, 1866, p. 253.

II. 0.4329 gram substance required 4.20 cc. N/I sulphuric acid.

	Calculated for NaC_6H_5	I.	Found.	II.
Na	23.04	22.68		22.36

Analysis of other preparations:

I. 0.3525 gram substance required 3.07 cc. N/I sulphuric acid.

II. 0.6843 gram substance required 6.47 cc. N/I sulphuric acid.

	Calculated for NaC_6H_5	I.	Found.	II.
Na	23.04	20.07		21.79

As sodium mercury phenyl, $\text{NaC}_6\text{H}_5\text{—Hg}(\text{C}_6\text{H}_5)_2$, has only 5.07 per cent of sodium, the above analyses show beyond doubt that the powder is sodium phenyl containing traces of mercury diphenyl. When sodium phenyl is exposed to the air on a filter-paper for a short time, it catches fire. As it is decomposed by water, the reactions were always carried out in the flask in which the sodium phenyl had been prepared, a calcium chloride tube being connected with the flask to prevent access of moisture.

Action of Sodium Phenyl on Ethyl Bromide and on Ethyl Iodide.

By carefully fractionating some low-boiling ligroin a product was obtained that boiled between 54° and 63° . 4.5 grams of mercury diphenyl were dissolved in about 50 cc. of this ligroin and converted into sodium phenyl in the usual way. The flask was then fitted with a dropping-funnel and connected with bromine absorption-tubes to absorb any ethylene formed. 2.5 grams of ethyl bromide were then added slowly through the dropping-funnel, the flask being kept cold by a freezing-mixture. The solution was then warmed to 40° to drive off any ethylene. On treating the bromine in the absorption-tubes with sodium hydroxide a few drops of ethylene dibromide, boiling at about 130° , were obtained. The ligroin solution was very carefully fractionated. After the main portion had distilled over there was a fraction at 80° to 90° , indicating that benzene was present, but this was not proved. The re-

mainder, by repeated fractionation, gave 2 grams of ethylbenzene, boiling at 130° to 135° . It was treated with nitric acid (20 per cent) in a sealed tube, at 160° , for six hours, and when the tube was opened a great deal of gas escaped. Not all of the ethylbenzene had been decomposed, but 0.5 gram of benzoic acid, melting at 119° , was obtained.

Sodium phenyl and ethyl bromide give, therefore, ethylbenzene and small amounts of benzene and ethylene.

Sodium phenyl (5 grams) suspended in ligroin (boiling-point 35° to 40°) was treated with the molecular quantity of ethyl iodide in absolute ether, the flask being kept cold. The flask was then connected with bromine absorption-tubes and warmed. Upon treating the bromine with sodium hydroxide only a drop or two of ethylene dibromide remained. When the ligroin solution was fractionated, 2 grams of ethylbenzene, boiling at 130° to 135° , were obtained. Heated with nitric acid, of sp. gr. 1.30, in a sealed tube for six hours at 160° , it gave 1.1 grams of pure benzoic acid melting at 122° .

Action of Sodium Phenyl on Isoamyl Iodide.

Sodium phenyl (5 grams) prepared in ligroin (boiling-point 55° to 60°) was treated with the molecular quantity of isoamyl iodide in absolute ether, the flask being well cooled by a freezing-mixture. When the reaction was over, the flask was connected with a bromine absorption-apparatus and warmed for some time. The bromine was treated with sodium hydroxide, and 1.1 grams of isoamylene dibromide, of boiling-point 62° to 64° at 15 mm., were obtained. When the ligroin filtrate was distilled a fraction boiling between 80° and 90° was obtained, indicating the formation of benzene in the reaction. Three grams of oil boiling between 175° and 200° were also obtained. This, by refractionation, gave 2 grams of isoamylbenzene, boiling at 190° to 195° (mostly at 193°).

Sodium phenyl and isoamyl iodide, therefore, give benzene, isoamylene, and isoamylbenzene.

Analysis of isoamylene dibromide:

0.8014 gram substance gave 1.3095 gram AgBr by Carius' method.

	Calculated for $C_8H_{10}Br_2$	Found.
Br	69.56	69.53

Analysis of isoamylbenzene :

0.2710 gram substance gave 0.8817 gram CO_2 and 0.2658 gram H_2O .

	Calculated for $C_{11}H_{14}$	Found.
C	89.09	88.73
H	10.91	10.90

Action of Sodium Phenyl on Benzyl Chloride.

When 5 grams of sodium phenyl, prepared in ligroin (boiling-point 55° to 60°), were treated with the molecular quantity of benzyl chloride there was a vigorous reaction. The ligroin filtrate gave, on distillation, 1 gram of a substance having the boiling-point 250° to 270° (diphenylmethane). At 300° to 320° a fraction passed over, which partly solidified. When dried, this melted between 115° and 125° and was shown to be stilbene by its conversion into stilbene dibromide (0.5 gram, melting-point 237° , being obtained). On refractionation, 0.8 gram of diphenylmethane, boiling at 260° to 265° , was obtained, which had the characteristic odor of this substance and which solidified when cooled.

Sodium phenyl and benzyl chloride, therefore, give diphenylmethane and stilbene.

Analysis of diphenylmethane :

0.2230 gram substance gave 0.7584 gram CO_2 and 0.1496 gram H_2O .

	Calculated for $C_{12}H_{12}$	Found.
C	92.80	92.76
H	7.20	7.45

Action of Sodium Phenyl on Brombenzene.

Sodium phenyl (5 grams) prepared in ligroin (boiling-point 60° to 80°) was treated with 7 grams (1 molecule) of brombenzene in ether. As the reaction was very vigorous, the flask was kept cool by a freezing-mixture. The solution, when distilled, gave 1.1 grams of diphenyl, boiling at 250° to 260° . This solidified and melted at 69° to 70° . Mixed with

a sample of Kahlbaum's diphenyl the melting-point was not lowered. There was a considerable fraction above 300° , but it was a resinous liquid that would not solidify.

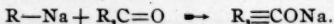
Analysis:

0.2397 gram substance gave 0.8203 gram CO_2 and 0.1410 gram H_2O .

	Calculated for $\text{C}_{12}\text{H}_{10}$	Found.
C	93.45	93.33
H	6.55	6.54

Action of Sodium Phenyl on Benzophenone.

Frey¹ found that when benzophenone is treated with molecular quantities of brombenzene and sodium in absolute ether, a quantitative yield of triphenylcarbinol is obtained. This has been confirmed. Similar carbinols are obtained when homologues of benzophenone and brombenzene are treated with sodium in the same way. Nef² explained this reaction on the basis of an intermediate formation of sodium phenyl, which is subsequently absorbed by the ketone, forming the sodium salt of the carbinol.



This is proved by what follows:

Mercury diphenyl (5 grams) was treated with sodium wire (5 grams) in benzene, and then 2 grams of solid benzophenone were slowly added with constant shaking and cooling. The mixture was treated with water and sodium carbonate, and the benzene solution then dehydrated and distilled. A residue was obtained which, when recrystallized from ligroin, gave 2.5 grams of triphenylcarbinol, melting at 162° . The ligroin filtrate was evaporated and the residue was digested with phenylhydrazine and absolute alcohol, 0.25 gram of benzophenonephenylhydrazone (melting-point 130°) being obtained. This shows that not all of the benzophenone had been acted upon by the sodium phenyl. A small amount of benzoic acid was obtained by acidifying the sodium carbonate solution. Two grams of benzophenone should give 2.86

¹ Frey: Ber. d. chem. Ges., 28, 2514.

² Nef: Ann. Chem. (Liebig), 308, 291.

grams of triphenylcarbinol. When we take into consideration the small amount of recovered benzophenone and the benzoic acid, the yield of triphenylcarbinol actually obtained, 2.5 grams, is nearly quantitative.

Analysis:

0.2382 gram substance gave 0.7644 gram CO_2 and 0.1357 gram H_2O .

	Calculated for $\text{C}_{19}\text{H}_{19}\text{O}$	Found.
C	87.62	87.52
H	6.21	6.33

The Action of Brombenzene and Sodium on Benzpinacone.

Benzpinacone (5 grams) in absolute ether was allowed to stand fourteen hours, in a sealed vessel, with 2.5 grams of brombenzene (1 molecule) and 1 gram of fine sodium wire (1 molecule). The reaction-product was poured into water and treated with sodium carbonate, but no benzoic acid was found in the alkaline solution. When the ether was evaporated and the residue recrystallized from alcohol, 2.4 grams of triphenylcarbinol, melting at 158° , were obtained. On adding water to the alcoholic filtrate a precipitate of benzhydrol was obtained, which, when recrystallized from ligroin, weighed 2.2 grams. It melted at 67° and gave a blood-red color with concentrated sulphuric acid. Benzpinacone is known to dissociate completely into benzophenone and benzhydrol at its melting-point, 185° , or on heating with alcoholic potash.¹ The above experiment shows that this dissociation takes place at room temperature. As fast as benzophenone is formed it reacts with the sodium phenyl (from brombenzene and sodium), giving triphenylcarbinol.

Analysis of benzhydrol:

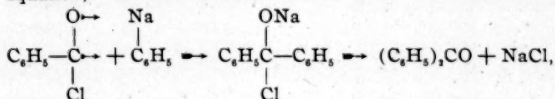
0.2542 gram substance gave 0.7908 gram CO_2 and 0.1494 gram H_2O .

	Calculated for $\text{C}_{13}\text{H}_{12}\text{O}$	Found.
C	84.73	84.84
H	6.58	6.53

¹ Thörner and Zincke: Ber. d. chem. Ges., 10, 1474.

Action of Sodium Phenyl on Benzoyl Chloride.

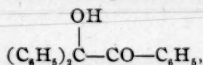
Mercury diphenyl (4.5 grams) was converted into sodium phenyl in benzene and treated with 1.5 grams of redistilled benzoyl chloride dissolved in absolute ether. The reaction was very vigorous. The mixture was poured into water and extracted with sodium carbonate, but very little benzoic acid was obtained. The benzene was distilled off. The residue, when recrystallized from alcohol, gave 1.5 grams of triphenylcarbinol melting at 156°. The alcoholic filtrate was boiled with phenylhydrazine, but no benzophenonephenylhydrazone was obtained. This shows that the benzophenone first formed from the benzoyl chloride and sodium phenyl, according to the equation,



reacts immediately with more sodium phenyl, giving triphenylcarbinol.

Action of Sodium Phenyl, and of Brombenzene and Sodium, on Benzil.

Eight grams of mercury diphenyl were converted into sodium phenyl in ligroin (boiling-point 60° to 80°) solution. Solid benzil (5 grams, melting-point 96°) was slowly added with constant stirring and cooling. The reaction took place instantly, a beautiful violet-colored solid being formed. The mass was poured into water, extracted with sodium carbonate, and the ligroin solution was dried with calcium chloride and distilled on a water-bath. When acidified, the sodium carbonate extract gave 1.5 grams of benzoic acid, which melted at 121° when recrystallized. The residue from the ligroin was treated with cold absolute alcohol; no residue of benzpinacone was left. The residue obtained by evaporating the alcohol consisted of a mixture of phenylbenzoin,¹

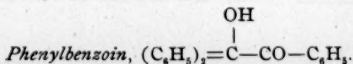


¹ This compound has since been described by Biltz in *Ber. d. chem. Ges.*, **32**, 650; see also Nef: *Ann. Chem. (Liebig)*, **308**, 291.

and triphenylcarbinol. These are readily separated by fractional crystallization from ligroin (boiling-point 60° to 80°) or alcohol, in which solvents the phenylbenzoin is by far the more soluble. Thus 1.5 grams of phenylbenzoin, melting-point 84° , and 1.3 grams of triphenylcarbinol, melting-point 155° , were obtained, besides a considerable amount of a resinous product that could not be made to crystallize.

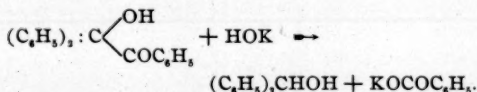
The same products were obtained when benzil was treated with brombenzene and sodium in absolute ether. To 5 grams of benzil in absolute ether were added 4 grams (1 molecule) of brombenzene and 1 gram (1 molecule) of fine sodium wire. The mixture was then kept sealed and cold for three days. The sodium was gradually attacked, an intensely colored, violet-blue solid being formed. Upon pouring the entire mixture into very dilute sulphuric acid, the blue color disappeared immediately. The ethereal solution was extracted with sodium carbonate, and 1 gram of benzoic acid, melting-point 119° , was obtained by acidifying the sodium carbonate solution. When the ethereal solution was dehydrated and distilled, a mixture of phenylbenzoin and triphenylcarbinol remained. This was extracted with ligroin (boiling-point 60° to 80°) and a residue of triphenylcarbinol was left, which, when recrystallized from alcohol, gave 1.2 grams of this substance, melting at 158° . From the ligroin extracts 1.4 grams of phenylbenzoin, melting-point 83° , were obtained.

Brombenzene and sodium, then, react with benzil exactly as sodium phenyl does, giving relatively the same yields of phenylbenzoin, triphenylcarbinol, and benzoic acid.



Phenylbenzoin is insoluble in water, but readily soluble in ether, alcohol, and hot ligroin. It crystallizes from the last solvent in radiating needles which melt at 87° . When the substance was dissolved in three parts of 25 per cent methyl alcoholic potash and warmed, a precipitate of potassium benzoate appeared in a short time. This was filtered off and converted into free benzoic acid, melting at 122° . The alcoholic filtrate gave, on addition of water, a white precipitate of benz-

hydrol, melting, when purified from ligroin, at 67° , and giving a blood-red color when warmed with concentrated sulphuric acid. The decomposition of phenylbenzoin by the action of the alkali may be represented as follows:



A blood-red color is observed when a solution of phenylbenzoin in concentrated sulphuric acid is warmed. This is also noticed in the case of benzilic acid, benzhydrol, etc., indicating dissociation into diphenylmethylenes¹ and other products. The following experiment confirms this view: Phenylbenzoin (0.5 gram) was heated to 240° in a long tube, in a stream of oxygen, for three hours. When cooled, the residue was treated with ether, and there remained a small amount of tetraphenylethylene, melting at 210° to 215° . On extracting the ethereal solution with sodium carbonate and acidifying the alkaline solution, 0.2 gram of benzoic acid, melting-point 120° , was obtained. After distilling off the ether the residue was digested for three hours with phenylhydrazine and alcohol; 0.25 gram of benzophenonephenylhydrazone, melting at 135° , was obtained. The melting-point remained unchanged when it was mixed with a sample of the substance prepared from benzophenone and phenylhydrazine.

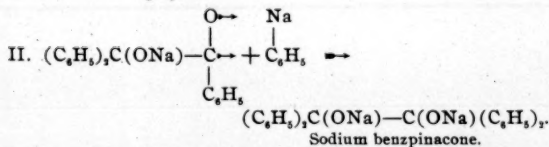
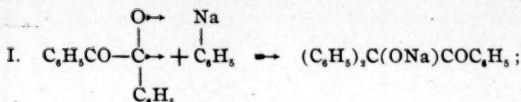
Analysis of the phenylbenzoin:

0.2040 gram substance gave 0.6224 gram CO_2 and 0.0992 gram H_2O .

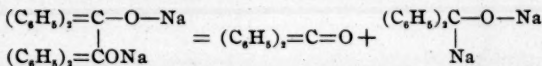
	Calculated for $\text{C}_{20}\text{H}_{16}\text{O}_2$	Found.
C	83.28	83.21
H	5.60	5.40

The action of sodium phenyl on benzil might result in the formation of two products, phenylbenzoin and benzpinacone, the course of the reaction depending upon whether 1 molecule of benzil reacts with 1 or 2 molecules of sodium phenyl.

¹ *Nef: Ann. Chem. (Liebig), 298, 241.*



The products actually obtained in the experiments described above are phenylbenzoin (I.), benzoic acid, and triphenylcarbinol, but no benzpinacone (II.). How are these facts to be explained? The sodium phenylbenzoin (I.) dissociates into diphenylmethylenes and benzoate of sodium, as has been made probable by the above experiments with phenylbenzoin. The diphenylmethylenes take up oxygen from the air, giving benzophenone, which naturally must react at once with the sodium phenyl present to give triphenylcarbinol. Furthermore, benzpinacone dissociates spontaneously into benzophenone and benzhydrol, as has been shown above in the action of sodium and brombenzene on this compound. It is, therefore, exceedingly probable that benzpinacone sodium (II.) dissociates spontaneously into benzophenone and its disodium salt,

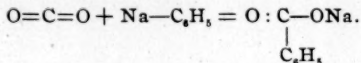


Hence, even if reaction II. takes place under the existing conditions, it is impossible to expect a formation of benzpinacone.

The Action of Sodium Phenyl on Carbon Dioxide.

Sodium phenyl was made in the usual way from 10 grams of mercury phenyl and 10 grams of sodium in ligroin (boiling-point 70° to 80°) solution. On passing dry carbon dioxide into the mixture a reaction took place with considerable evo-

lution of heat. The reaction-product was poured into water and extracted with sodium carbonate. The alkaline solution gave, when acidified, 6 grams of crude benzoic acid, which, by recrystallization, gave 4.5 grams of the pure acid, melting at 122° . When mixed with a sample of benzoic acid, the melting-point was not changed. The theoretical yield is 6.9 grams of benzoic acid. This experiment was tried three times, and in every case good yields were obtained. The action of carbon dioxide on sodium phenyl is analogous to that of carbon dioxide on sodium acetylenes,¹ sodium zinc alkyls,² and on phenyl aluminium chloride:³



Kekulé⁴ found that carbon dioxide acting upon brombenzene and sodium gave benzoic acid, diphenyl, and small amounts of benzophenone and phenyl benzoate. This shows that in a mixture of brombenzene, sodium, and carbon dioxide, the sodium first acts upon the brombenzene to give sodium phenyl. Part of the sodium phenyl reacts with unchanged brombenzene to give diphenyl, while another part reacts with the carbon dioxide to give benzoic acid.

Action of Sodium Phenyl on Chlorcarbonic Ether.

Wurtz⁵ discovered that brombenzene, chlorcarbonic ether, and sodium amalgam give benzoic ether, and other homologues of brombenzene give corresponding homologues of benzoic ether.⁶ The yields in all cases are very poor, as the chlorcarbonic ether gives a great deal of carbon monoxide and diethyl carbonate; mercury phenyl, etc., is also formed. It is now obvious that the sodium first reacts with the brombenzene, forming sodium phenyl, which then combines with the chlorcarbonic ether to give benzoic ether and sodium chloride.

¹ Lagermark : Ber. d. chem. Ges., 12, 854; Nef : Ann. Chem. (Liebig), 308, 281.

² Wanklyn : Ann. Chem. (Liebig), 107, 125; 111, 234.

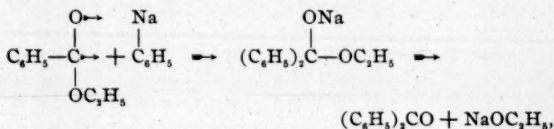
³ Friedel and Crafts : Ann. chim. phys., [6], 14, 441.

⁴ Kekulé : Ann. Chem. (Liebig) 137, 178.

⁵ Wurtz : *Ibid.*, Suppl., 7, 125.

⁶ Kekulé : Ber. d. chem. Ges., 7, 1007; Jacobson : *Ibid.*, 17, 2374; Nahnsen : *Ibid.*, 17, 2192.

Five grams of mercury diphenyl in ligroin (boiling-point 60° to 80°) were converted into sodium phenyl. Three grams of chlorcarbonic ether in absolute ether were then slowly added, the solution becoming quite warm. When water was added and the solution extracted with sodium carbonate, no benzoic acid was obtained on acidifying the alkaline solution. The ligroin solution, when dried and distilled, gave only a small amount of benzoic ether, boiling at about 210° . It was identified by its odor and by conversion into benzoic acid (melting-point 118°) by means of methyl alcoholic potash. The residue from which the benzoic ether had been distilled was crystallized from alcohol, and 1.1 grams of triphenylcarbinol, melting at 158° , were obtained. The alcohol filtrate was boiled with phenylhydrazine, but no benzophenonephenylhydrazone was obtained. Sodium phenyl and chlorcarbonic ether, as is obvious from the preceding, give very little benzoic ether. The benzoic ether first formed must, therefore, react further with sodium phenyl, as follows:



and the resulting benzophenone then reacts with more sodium phenyl, forming triphenylcarbinol. This is proved by the following experiment:

Action of Brombenzene and Sodium on Benzoic Ether.

When a solution of 3 grams benzoic ether and 6 grams brombenzene in absolute ether was allowed to stand sealed twenty-four hours with 2 grams of sodium wire, no metal was left. The solution was colored deep-blue and a blue precipitate covered the bottom of the vessel.

The reaction-products were found to be 1.4 grams triphenylcarbinol, melting at 158° , 0.2 gram of benzoic acid, 0.3 gram of triphenylmethane, melting-point 92° to 93° , and benzophenone, which was identified by means of its phenyl-

hydrazone (0.42 gram melting at 135°). In a second experiment, where the same amounts of benzoic ether and brombenzene but more sodium, 3 grams instead of 2 grams, were used, no benzoic acid was obtained. The other products were triphenylcarbinol, 1.2 grams, triphenylmethane, 0.65 gram, and benzophenone, 1.4 grams, melting at 48° to 49° . The triphenylcarbinol was separated from the other two products by crystallization from alcohol. The alcoholic filtrate, in the second experiment, gave, on fractional distillation, 2.4 grams of material boiling between 280° to 350° —a mixture of benzophenone and triphenylmethane. This was partially separated by fractional distillation and further by fractional crystallization.

Action of α -Bromnaphthalene and Sodium on Benzophenone.

α -Naphthyldiphenylcarbinol.—When 10 grams of benzophenone, 12 grams of α -bromnaphthalene, and 5 grams of fine sodium wire in absolute ether stood one week in a sealed vessel, and the mixture was treated with water and extracted with potassium hydroxide, a small amount of benzoic acid was obtained. The ether was distilled off, and the residue, by distillation with steam, gave 1 gram of naphthalene, melting-point 80° . The part not volatile with steam was dissolved in ether and ligroin, and after long standing a substance crystallized out which melted at 135° when recrystallized from ether and ligroin. It was easily soluble in ether and alcohol, but insoluble in water and ligroin.

The same compound, α -naphthyldiphenylcarbinol, was formed when 5 grams of brombenzene, 5 grams of α -naphthylphenylketone, and 2 grams of sodium wire in absolute ether stood two days. When the ethereal solution was treated with water and extracted with sodium carbonate no acid was obtained. The ethereal solution was dehydrated and distilled. The residue, crystallized from ether and ligroin, gave α -naphthyldiphenylcarbinol, melting at 135° . When samples of the two products were mixed, the melting-point was not changed.

Analysis:

I. 0.1721 gram substance gave 0.5589 gram CO_2 and 0.0952 gram H_2O .

II. 0.1912 gram substance gave 0.6192 gram CO_2 and 0.1023 gram H_2O .

	Calculated for $\text{C}_{25}\text{H}_{18}\text{O}$.	I.	Found.	II.
C	89.00	88.57		88.32
H	5.86	6.14		5.95

Action of p-Bromtoluene and Sodium on Benzophenone.

Benzophenone (5 grams), *p*-bromtoluene (5 grams), and sodium (2 grams) in absolute ether stood two weeks in a sealed vessel. The solution became intensely blue and a blue precipitate was formed. When the solution was treated with water and extracted with sodium carbonate no acid was found. When the ether was evaporated off and the residue dissolved in ligroin and ether, a substance at length crystallized out which melted at 74° when recrystallized from ligroin. This is *p*-tolylidiphenylmethane.¹

The same substance was formed when 5 grams of *p*-tolylphenylketone, melting-point 58° , 3 grams of brombenzene, and 2 grams of fine sodium wire in absolute ether, were allowed to stand one week. The mixture was poured into water and extracted with sodium carbonate, but no organic acid was found. When the ether was evaporated off and the residue distilled with steam, about 1 gram of brombenzene was recovered. The residue was distilled in a vacuum and 1 gram of a liquid boiling at 195° to 200° , at 12 mm., was obtained, which, upon standing, gradually solidified. This was crystallized from ether and ligroin and gave 0.5 gram of *p*-tolylidiphenylmethane, melting at 74° . When samples of the two products were mixed, the melting-point was not changed. The yield is very poor in both cases and apparently no *p*-tolylidiphenylcarbinol is formed.

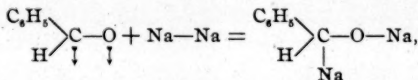
Five-tenths gram of the *p*-tolylidiphenylmethane was heated in a sealed tube with nitric acid (20 per cent) for twenty-four hours, at 150° to 160° . When the tube was opened a great

¹ Cf. E. and O. Fischer: Ann. Chem. (Liebig), 194, 263.

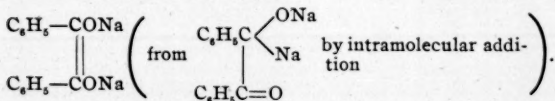
deal of gas escaped. The product was then taken up in ether and extracted with sodium carbonate. When this alkaline solution was acidified, 0.2 gram of *p*-triphenylcarbinolcarboxylic acid,¹ melting-point 206°, was obtained; this was further identified by its very insoluble barium salt.

The Action of Sodium on Ketones.

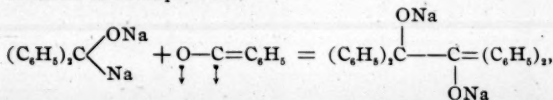
The action of sodium on carbonyl compounds, as aldehydes, ketones, acid chlorides, and acid esters has been discussed by Nef. In Liebig's *Annalen der Chemie*, 308, 283 to 292, he presents the view that at first disodium addition-products,



are formed which immediately react further with a second carbonyl group present in the same or in another molecule. Thus the benzil sodium of Beckmann and Paul was proved by him to be sodium dioxystilbene,

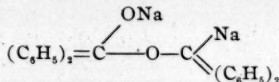


Similarly, benzophenone disodium must act with a second molecule of benzophenone,



Sodium benzpinacone.

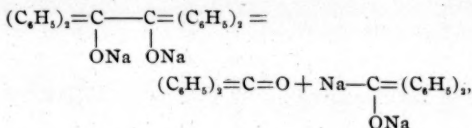
to give sodium benzpinacone. Beckmann and Paul² ascribe the following very improbable constitution to the product obtained by them by the action of sodium on benzophenone,



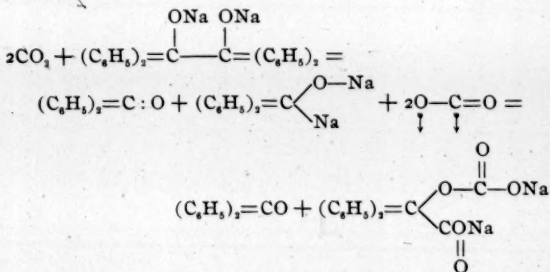
¹ Oppenheimer: Ber. d. chem. Ges., 19, 2029.

² Ann. Chem. (Liebig), 266, 9 (1891).

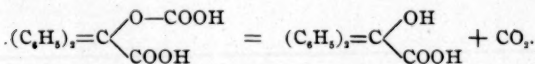
Their salt gives, on treatment with water, benzpinacone and equal molecules of benzophenone and benzhydrol. The relative amounts obtained vary tremendously according to the amount of water used and possibly also according to other conditions. Furthermore, their dry salt gave, on treatment with dry carbon dioxide, equal molecules of benzophenone and benzoic acid. These results are self-evident on the basis of the ideas of Nef. We have but to conclude that sodium benzpinacone dissociates at ordinary temperatures into benzophenone and benzophenone disodium,



as is probable from the facts known regarding benzpinacone itself, as well as from the experiments described above with benzpinacone, sodium, and brombenzene. We must, in consequence, expect to obtain from this salt, by the action of water, varying quantities of benzpinacone and equal molecules of benzhydrol and benzophenone. With dry carbon dioxide (provided the non-dissociated salt does not act *per se*) we must obtain equal molecules of benzophenone and carboxylated benzilic acid, as follows:



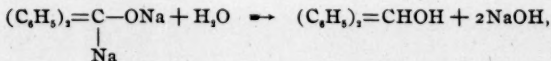
The carboxylated benzoic acid must lose carbon dioxide readily,



The formation of equal molecules of benzophenone and of benzoic acid in this reaction as observed by Beckmann and Paul is, therefore, easily understood. Furthermore, in continuing experiments in this direction, we have been able to prove that a carboxylated benzoic acid (oily acid, readily losing CO_2) is actually formed, thus establishing the correctness of the above conclusions.

Action of Sodium on Benzophenone.

Benzophenone (melting-point 48° to 49° , 5 grams) in absolute ether was treated with 2 grams of sodium (3 atoms) for three days. The solution became intensely blue and a blue solid was formed. The mixture was poured into water and extracted with sodium carbonate, but no organic acid was found. Upon evaporating the ether and crystallizing the residue from alcohol, 3 grams of pure benzopinone (melting-point 185° to 186°) were obtained. By boiling the alcoholic filtrate with phenylhydrazine, only a small quantity of benzophenonephenylhydrazone was obtained. Upon distilling off the phenylhydrazine with steam and recrystallizing the residue from ether and ligroin, 1.6 grams of benzhydrol, melting at 66° , were obtained. Whether the large quantity of benzhydrol formed came from the action of water on benzophenone disodium,



or was formed by the reduction of the benzophenone by the nascent hydrogen liberated from the excess of sodium when the reaction-mixture was poured into water, is not certain.

Analysis of benzopinone:

0.2252 gram substance gave 0.7001 gram CO_2 and 0.1225 gram H_2O .

	Calculated for $C_{28}H_{22}O_2$	Found.
C	85.20	84.79
H	6.07	6.04

The Action of Sodium and Carbon Dioxide on Benzophenone.

A flask containing 5 grams of benzophenone and 7 grams (11 atoms) of fine sodium wire in 100 cc. absolute ether was attached to a return-condenser through which was passed a tube leading in well-dried carbon dioxide. The flask was well shaken, and the benzophenone sodium was evidently acted on as fast as formed, for the blue color was never very intense. The mixture was poured into a separatory-funnel and water was added to dissolve the sodium salts. When the water solution was acidified, an oily acid, probably carboxylated

benzilic acid, $(C_6H_5)_2C \begin{matrix} \diagup OCOOH \\ \diagdown COOH \end{matrix}$, which did not change as

long as the water was cold, was precipitated; but when the water was warmed the oil decomposed with a lively evolution of carbon dioxide, forming 3 grams of pure benzilic acid (theory 5.5 grams) that melted at 149° to 150° .

Analysis:

0.2345 gram substance gave 0.6330 gram CO_2 and 0.1132 gram H_2O .

	Calculated for $C_{14}H_{10}O_4$	Found.
C	73.65	73.40
H	5.31	5.35

On the Isomeric Diacetyldioxystilbenes.

During the progress of Nef's work on sodium benzil and its conversion into diacetyldioxystilbene, two isomeric diacetyldioxystilbenes, melting at 110° and 153° , respectively, were independently discovered by Thiele.¹ We have repeated Thiele's experiments and proved that the lower melting isomer is identical with Nef's diacetyldioxystilbene, which, when perfectly pure (recrystallization from ether), melts at 118° .

The diacetyldioxystilbene of Nef has been examined crystallographically by Bowman in Groth's laboratory.² Two

¹ Thiele: Ann. Chem. (Liebig), 306, 142; cf. Nef: *Ibid.*, 308, 290.

² Nef: Ztschr. für Krystallographie; cf. Ann. Chem. (Liebig), 308, 290.

preparations, one made by Nef's and the other made by Thiele's method, were therefore submitted to Dr. O. C. Farrington, of the Field Columbian Museum, for comparison. His report (July 15, 1899) is as follows :

"The crystals of the two lots submitted by Mr. Acree proved to be entirely similar. They are all alike in habit, being made up of a short, stout prism, truncated to a somewhat tabular form by the prominent orthopinacoid. The orthodome $d(101)$ is also a prominent face, while $c(001)$ appears as a narrow face truncating the edge $a d$.

"As crystals of lot No. 1 had previously been measured by Bowman (*loc. cit.*) I confined my measurements to those of lot No. 2. Few faces on these crystals were suited to accurate measurement, oscillatory combinations usually causing a line of signals several degrees in length. The following measurements made on two different crystals may, however, be deemed characteristic. For comparison the values of similar angles calculated from Bowman's ratios are given :

	Measured.	Mean.	Calculated.
$a' : c = (100) : (001)$	86° 46'		
	86° 40'	86° 47'	86° 56'
	86° 55'		
$a : m = (100) : (110)$	70° 22'		
	70° 30'	70° 20'	70° 30'
	70° 8'		
$m' : m'' = (110) : (110)$	39°	39° 4'	39°
	39° 8'		
$m : n = (110) : (120)$	9° 20'		
	9° 40'	9° 30'	9° 28'
$a : d = (100) : (101)$	42° 25'	42° 25'	42° 25'

"The form K(102) noted by Bowman was not observed on the crystals. The calculated and measured values for the angles quoted above agree so closely that there can be no doubt that the crystals all have the same form."

Analysis of the diacetyldioxystilbenes, obtained by Thiele's method :

I. 0.2198 gram substance (melting-point 107° to 108°) gave 0.5837 gram CO₂ and 0.1139 gram H₂O.

II. 0.2191 gram substance (melting-point 118°) gave 0.5811 gram CO_2 and 0.1102 gram H_2O .

III. 0.2188 gram substance (melting-point 153°) gave 0.5848 gram CO_2 and 0.1032 gram H_2O .

	Calculated for $\text{C}_{19}\text{H}_{15}\text{O}_4$	I.	Found. II.	III.
C	72.92	72.43	72.34	72.89
H	5.46	5.76	5.59	5.24

I wish to thank Dr. J. U. Nef for the great inspiration and encouragement he has given me during this work.

Contributions from the Chemical Laboratory of the Massachusetts Institute of Technology.

XLI.—THE ACTION OF ZINC ON TRIPHENYL-CHLORMETHANE.

[SECOND PAPER.]

BY JAMES F. NORRIS.

A reply by M. Gomberg in the April number of this JOURNAL to a paper under the above title by Miss L. R. Culver and myself¹ contains a number of criticisms of our statements and experimental work. The reply was evidently written after a very hasty reading of the experimental evidence put forward by us, and, consequently, I hope to show that the criticisms of our experiments are not well founded, and that the suggestions put forward in explanation of our results are not in accord with the facts.

Action of Sodium.

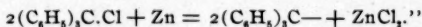
M. Gomberg takes exception to our statement that "It was shown in the same paper that, under certain conditions, triphenylchlormethane reacts with sodium, contrary to the observations of Gomberg." This statement is true, for it was shown that while sodium is inactive toward triphenylchlormethane, dissolved in dry ether, it did react with the halogen compound if brombenzene were present. As with other halides of this type, a catalytic agent is necessary to bring about a reaction between triphenylchlormethane and sodium. Bromben-

¹ This JOURNAL, 29, 129.

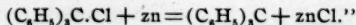
zene played this part in our experiments. We have accumulated a number of facts on the action of sodium on triphenylchloromethane, which have not been published as yet. In dry ether there is no reaction after a number of days, but if a little ethyl acetate is added a reaction soon begins between the metal and the halogen compound. In this respect triphenylchloromethane is like other alkyl halides.

Is the Reaction Quantitative?

We said in our paper, "At the beginning of the work it was found that the statement of Gomberg was incorrect, that when the reaction between zinc and triphenylchloromethane took place in benzene solution the metal reacted quantitatively according to the following equation :



In reply, Gomberg says: "In turning to the reference made by the authors to my paper in respect to this question, we see at once that there is no such statement either made or implied. We read: 'The zinc removes the halogen from triphenylchloromethane quantitatively.'"¹ I think that a reference to Gomberg's original paper will lead one to the conclusion stated in our sentence. It was there stated that zinc removes the halogen quantitatively, and this was shown by determining the chlorine in the zinc chloride formed. The amount of chlorine removed as zinc chloride was stated in the tabulation of the results. This experiment was faulty. If all the chlorine had been converted quantitatively into zinc chloride, as claimed by Gomberg, the only thing left was, of course, the two $(\text{C}_6\text{H}_5)_3\text{C}^-$ groups. This is expressed, as far as I can see, by the equation written by us. That Gomberg was of this opinion is shown by the following statement which appears later in the same paper (page 768): "The action of zinc results, as it seems to me, in the mere abstraction of the halogen, leaving the free radical,



¹ J. Am. Chem. Soc., 22, 761 (1900).

Formation of the Double Salt.

By putting together two sentences from one paragraph, without indicating that anything stood between them, Gomberg makes us say what we did not say, and then proceeds to answer it. He says that Norris and Culver say: "During the course of the reaction a thick, dark mass separates, which Gomberg assumed to be a compound of benzene and zinc chloride. Gomberg, in a later paper, briefly mentions this mistake, but he has reported no further work to test this point." In our paper, after the first sentence just quoted, comes an account of Gomberg's method of determining the chlorine removed by the zinc. Then comes the following: "The single quantitative experiment on which Gomberg based his theory in his first paper was, therefore, manifestly incorrect, and there were no data as to the amounts of zinc and triphenylchlormethane which entered into the reaction. Gomberg, in a later paper, mentions this mistake, but he has reported no further work to test this point." It is evident that reference was made to the relation between the amounts of zinc and triphenylchlormethane that reacted with one another, and we repeat that our paper furnishes the only data available on this important point. Gomberg also objects to the word "single" in the above sentence, and says that he made three determinations of the chlorine removed. He can hardly consider an endeavor to get checking results in a quantitative determination as three quantitative experiments in support of his conception of trivalent carbon.

Action of Zinc in Ethyl Acetate.

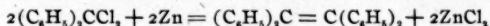
Gomberg then takes exception to our statement: "As triphenylmethane, triphenylcarbinol, and an oil are at times present, an investigation of the cause of the formation of these substances must be made before the reaction, which is evidently a very complex one, can be exactly interpreted." He states that he had already shown that these substances are formed, but admits that he had not explained the cause of their presence. He says: "It remains, however, to determine whether these products are formed before or during the

oxidation of the unsaturated hydrocarbon." It was in an attempt to find an explanation of these facts, which it seemed to us must be understood before the reaction is cleared up, that we undertook a quantitative study of the reaction.

The criticisms of our experimental results and their interpretation, which then follows, seem to me to be wholly valueless. In regard to the large amount of zinc which we found was used in the reaction, Gomberg suggests that the conclusions drawn are falsified by the fact that zinc oxide was produced, as he says, "by the direct oxidation of the metal by the oxygen, under the influence of the different products present." The amount of zinc oxide formed was determined and found to be so small that it was not taken into account in the calculation of the results. It is also suggested in a footnote that zinc may be soluble in a solution of zinc chloride in ethyl acetate. In one experiment reported in our first paper, Gomberg's equation required for the triphenylchlormethane used 0.127 gram of zinc, whereas 0.252 gram entered into the reaction. According to Gomberg, a 10 per cent solution of zinc chloride in ethyl acetate dissolved but 0.035 gram of zinc (from how much is not stated) in five days and 0.066 gram in two weeks. In our experiment about 1 gram of zinc was present during the reaction, which was shown to be complete in ten days. The dilution was such that had all the chlorine been present at the end of the reaction as zinc chloride, the solution would have been one of about 1 per cent. It seems to me, therefore, that Gomberg has suggested no explanation which can be considered helpful, of the large amount of zinc which reacts with triphenylchlormethane. The fact that so much of the metal does enter into the reaction is of vital importance, when the reaction is to be completely understood.

While zinc may be dissolved by a solution of the ordinary zinc chloride dissolved in ethyl acetate, as claimed by Gomberg, our experimental results lead us to the conclusion that it is not acted upon by the pure chloride produced by the action of the metal on an organic chloride dissolved in pure ethyl acetate. An experiment with benzophenone chloride gave positive evidence on this point. About 9 grams of the

chloride, dissolved in 25 cc. of ethyl acetate, were shaken with 10 grams of zinc for a number of days. As long as any benzophenone chloride was present, the solution had a dark green color due to the presence of a compound of the ketone chloride and zinc chloride. After shaking for 5 days the reaction was complete, as the solution had become colorless. The mixture was shaken for eight more days in order to determine whether the zinc present (about 7.6 grams) would be dissolved by the zinc chloride formed in the reaction (about 5 grams). A determination of the zinc which remained showed that 2.47 grams had reacted, whereas the amount required by the equation



was 2.56 grams. It is evident, therefore, that the zinc chloride had dissolved no zinc, and that Gomberg's explanation of the large amounts used in our experiments with triphenylchlormethane is not in accord with the facts.

Absorption of Oxygen.

Gomberg's statement that the conclusion drawn by us from the large amount of oxygen absorbed is invalidated by the fact that zinc oxide was formed, is again not in accord with the facts. In some of the experiments the amount of zinc oxide formed was determined. Experiment 4, given in our first paper, can be taken as an example. The amount of zinc used was 0.417 gram, whereas 0.248 gram is required by Gomberg's statement, that the chlorine is removed quantitatively as zinc chloride. In this experiment 0.098 gram of oxygen was absorbed, whereas the peroxide obtained contained only 0.046 gram. The zinc oxide was determined and found to be 0.012 gram. This contains about 0.003 gram of oxygen, which is such a small quantity that it is almost negligible in comparison with the difference between the oxygen in the peroxide and the total absorbed in the reaction—0.052 gram. The data about the quantities of zinc oxide formed were not published in our former paper, as the amounts were small, and as it had not been determined whether the oxide was formed during the oxidation of the unsaturated com-

pounds, or when the solution was brought into contact with the moisture of the air. The fact remains that the amounts were so small that it was not considered necessary to introduce them into the calculations. The criticism of Gomberg of our results is, therefore, of no value.

Gomberg also points out that no reference was made to his observation that triphenylmethyl absorbs about 14 per cent more than the theoretical quantity of oxygen in going over to the peroxide. Our observations are of an entirely different nature, for in our experiments from two to three times the theoretical amounts were absorbed by the solution in which the reaction between triphenylchlormethane and zinc took place. We were evidently dealing with a different reaction from the one studied by Gomberg, when the excess was only about one-seventh of the whole. The results do warrant us, therefore, in believing that we have observed a reaction overlooked by Gomberg.

The Yield of the Peroxide.

Gomberg points out under this title that our results in different experiments are not uniform, that we did not obtain the same amount of peroxide in each case, and that, as a consequence, no conclusions can be drawn. The point is not well taken. While the yield of the peroxide did vary greatly, this variation was always accompanied by a corresponding variation in the amounts of zinc used and the other products formed. As the percentage of peroxide decreased the amounts of zinc used and carbinol formed increased. The results are not chance ones, and the variation of which Gomberg complains takes place with all the substances involved, in such a way that they help us in interpreting the reaction.

Gomberg states gratuitously (page 370): "As the authors did not assure themselves that the action of zinc on triphenylchlormethane was carried to the end, etc." It is clearly stated in our first paper how we determined when the reaction was complete. In describing our experiments we said: "At first the gas was absorbed quite rapidly, but toward the end of the experiment the absorption was very slow.

The yellow color of the solution slowly decreased until it had almost entirely disappeared, when the reaction was complete. When the absorption ceased the flask was removed and the zinc left determined." The data on the rate of absorption in each experiment reported are at hand. When, after standing twenty-four hours, there was no further decrease in the volume of oxygen, it was plain that the reaction was complete. In our experiments the reaction was allowed to proceed for eight to fourteen days. Gomberg also asks, "What precautions were taken to prevent the zinc from becoming coated with a layer of the insoluble triphenylmethyl peroxide?" This possible source of error was, of course, looked after.

A criticism then follows which could not have been written had our quantitative results been examined. Gomberg, in attempting to explain the presence of triphenylcarbinol, makes this statement: "Still, as the authors did not assure themselves that the action of zinc on triphenylchlormethane was carried to the end, *i. e.*, that all the halogen compound was attacked by the metal, and as they treated the reaction-product with water, it is evident that any unchanged triphenylchlormethane would be converted by this treatment into the triphenylcarbinol." According to this "explanation" of the high yield of carbinol, the latter is produced by the action of water on the triphenylchlormethane which had not entered into reaction with zinc. If this view is correct, it follows that less than the theoretical amount of zinc also reacted, as it is assumed that the reaction was incomplete. The results published in our paper prove that this is not the case. The largest yield of carbinol was obtained when the *largest* amount of zinc was used. In Experiment 2, described in our paper, nearly twice the amount of zinc required by Gomberg's equation entered into the reaction, and yet a large percentage of carbinol was obtained. The experimental results show that Gomberg's attempt to find a source of error in our work is unsuccessful.

From the above it will be seen, I think, that Gomberg's criticisms and suggestions are entirely inconsistent with one another. In order to explain the large amount of zinc used,

he suggests that the metal was dissolved by the zinc chloride formed in the reaction, and was oxidized to zinc oxide by the oxygen present. In order to explain the formation of the large amount of triphenylcarbinol found in the same experiment, when the zinc used is far in excess of the theoretical amount calculated from his conception of the reaction, he assumes that the reaction was incomplete, and that the metal was coated with the precipitated peroxide, and so prevented from entering into reaction. These views could not have been put forward if the results published by us had been thoroughly studied.

In conclusion, I can say that I believe that not a single point brought out by Gomberg in his last paper has a bearing on our experimental work and conclusions, and that I still believe that these results show that in ethyl acetate solution the reaction between zinc and triphenylchlormethane is a very complicated one.

BOSTON, April 8, 1903.

REVIEWS.

PRINCIPLES OF INORGANIC CHEMISTRY. By HARRY C. JONES, Associate Professor of Physical Chemistry in Johns Hopkins University. New York: The Macmillan Co. 1903. pp. xx + 522. Price, \$4.00.

The qualities of the style of the prolific and enthusiastic writer of this work are too well known to need detailed review at this time. The work under review has the highly commendable object of presenting the facts of inorganic chemistry in a systematic manner, with the help of the recent laws and theories of physical chemistry. As the master, van't Hoff, has pointed out, inorganic chemistry acquires its greatest interest and significance when treated in this way. The problem is not altogether an easy one, because it involves the interweaving of a double classification. In this work the writer has wisely used the periodic system as the first basis of the arrangement, and has introduced the modern ideas where they seemed most likely to be useful. The periodic system itself is not explained until three typical elements, oxygen, hydrogen, and chlorine, have been studied in detail. The discussion of Faraday's Law, the Phase Rule, and the Law of "Mass-Action" are taken up at the first opportunity, so that they may be useful in classifying subsequent facts. Of-

ten the generalization is very opportune, but occasionally it must be confessed that the bodily transfer of pages from the author's work on physical chemistry is evident.

As every one knows, it is almost impossible to write a book which shall be free from errors, and this one shares the common lot. On page 10, Proust, of Angers, is spoken of as an Englishman; on page 86, an impracticable apparatus is depicted, and there are a few mistakes in proof-reading which affect important figures or symbols. A more serious defect than these trivial points seems to me, however, an occasional lack of clearness in the distinction between fact and hypothesis. This is perhaps best illustrated by a quotation from page 71: "The hypothesis of Avogadro has been confirmed by such an abundance of subsequent work, in so many directions, that it is now placed among the well established laws of nature." This somewhat doubtful attitude is not merely a matter of words; it seems to penetrate the thought as well, at times. One of its results is the occasional tendency to overlook the exceptions or difficulties attending many hypotheses; for example, a beginner would probably leave the book with the idea that the hypothesis of ionization is a perfect theory which leaves nothing unexplained. The reviewer does not mean hereby to discourage the use of hypotheses but merely wishes to emphasize the importance of realizing their hypothetical nature.

One sees with regret the retention of Trevor's polyglot nomenclature for degrees of freedom, and hopes that in a subsequent edition the purely Latin form of this nomenclature suggested by the reviewer five years ago and more recently adopted by Trevor himself, may be substituted. On the other hand, one sees with pleasure the adoption of oxygen as the standard of atomic weights, and in many other cases, where there is a choice between two paths, the right one seems to have been taken. There is but one index, giving both authors and subjects, and no references to the original sources of the information are recorded. One unusual omission should perhaps be mentioned, the specific gravities of solids and liquids are very rarely stated.

All things considered, the book is interesting and will probably be useful and render inorganic chemistry good service by inciting the inorganic chemist to think about the general bearings of the facts with which he deals.

T. W. R.

ÜBUNGSBEISPIELE FÜR DIE ELEKTROLYTISCHE DARSTELLUNG CHEMISCHER PRÄPARATE, ZUM GEBRAUCH IM LABORATORIUM FÜR CHEMIKER UND ELEKTROCHEMIKER. Von DR. KARL ELBS, o. Professor und Direktor des Laboratoriums für physikalische und organische Chemie an der Universität Giessen. Mit 8 Abbildungen im Text. Druck und Verlag von Wilhelm Knapp. Halle, a. s. 1902. pp. 100.

This little book gives a clear and concise account of methods for preparing electrolytically some of the more important inorganic and organic compounds. The first part deals with the electrical apparatus which is necessary for such work. This is followed by a description of methods for the preparation of a number of salts of sodium and potassium.

The latter part of the book, dealing with organic synthesis, is much wider in its scope. As is well known, electrolytic synthesis in organic chemistry has to do chiefly with the aromatic compounds. This fact is made evident by the little work in hand, which will doubtless prove to be useful in connection with the new and interesting applications of electrochemistry and organic chemistry.

H. C. J.

HANDBUCH DER ELEKTROCHEMIE. Bearbeitet von PROF. DR. BORCHERS, Aachen; PRIVATDOCENT DR. E. BOSE, Göttingen; PRIVATDOCENT DR. H. DANNEEL, Aachen; PROF. DR. R. ELBS, Giessen; PROF. DR. F. KÜSTER, Clausthal; BERGINGENIEUR F. LANGGUTH, Meckernich; PROF. DR. W. NERNST, Göttingen; und PROF. DR. H. STOCKMEIER, Nürnberg. Verlag von Wilhelm Knapp. Halle, a. s. 1903.

According to the announcement the "Handbuch der Elektrochemie" is to be an elaborate treatise on this very important chapter in physical chemistry. The work aims to cover the whole field of electrochemistry from both the scientific and technical standpoints, and from the staff of authors, will undoubtedly be a masterpiece.

The parts which have appeared thus far are: "Elektromagnetische Aufbereitung," by F. Langguth, and "Specielle Elektrochemie," by H. Danneel. The "Theoretische Elektrochemie," will be written by Prof. Dr. W. Nernst; "Messkunde," by Dr. E. Bose; "Elemente und Akkumulatoren," by Prof. Dr. K. Elbs; "Elektroanalyse," by Prof. Dr. F. Küster; "Anorganisch-elektrochemische Technik," by Prof. Dr. W. Borchers; "Organisch-elektrochemische Technik," by Prof. Dr. K. Elbs; and "Galvanotechnik," by Dr. H. Stockmeier.

The work ought to be in every public scientific library, as well as in the libraries of individuals who are interested in any phase of electrochemistry.

H. C. J.